ELECTROPHYSIOLOGY OF RICE ROOTS

by

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PREFACE

All the experimental work in this thesis was carried out in the Biophysics laboratory of the Physics Department, University of Tasmania, from January, 1982 until August 1984, under the supervision of Dr. B.I.H. Scott. Work was carried out with financial support from Australian Development Assistance Bureau under a Colombo Plan Scholarship until March 1984 and from The University of Tasmania until February 1985 for which the author is most grateful.

Part of the work was presented to the Ninth Annual Meeting Proceedings of the Australian Society for Biophysics by the author and Dr. B.I.H. Scott.

SUMMARY

The electrophysiology of rice (*Oryza sativa* cv. Calrose) roots has been investigated in both intact and excised tissues. Mathematical procedures for measuring ion fluxes across the plasmalemma, the tonoplast and the xylem has been developed based on the Pitman model of ion transport. The mathematical analysis has been tested by means of the radioactive tracers. The method allows one to estimate ion fluxes in the longitudinal and radial directions and the efflux of ions from the xylem vessels to the symplast. The movements of K^+ ions were followed in this study, using ^{86}Rb as well as ^{42}K.

A study has been made of primary roots grown under the same salt concentration thoughout their life, using a mature portion of the root where ionic fluxes are changing relatively slowly with time. It was found that ion exchange between cortical cells in the longitudinal direction was not more than 5% of the radial transport into the xylem. This was also true when the megnitude of apoplastic transport was compared to that of symplastic transport, regardless of whether or not the Casparian strip had developed at the endodermal cell walls. It was found that the net absorption into this region of the root from the external medium was small. Hence, most of ions which were transported into the shoot were from reserves in the cell vacuoles. Consequently, there was a gradual loss of K^+ ions from the studied portion, suggesting the loss of absorption capability of cortical cells after being fully differentiated. There was also evidence showing the formation of large air spaces at the mature root region.

In the transport system, it was found that an appreciable amount of K^+ ions in the xylem vessels was re-absorbed by the cells surrounding them. Re-translocation of ions from the shoot via the phloem to the root was also found in this study. Most of this transport was found to be to the tip region rather than to the mature cell region.

The application of the Ussing-Teorell equation showed that there were K^+ inward pumps locating at the plasmalemma and at the site of delivery of ions into the xylem. These pumps disappeared in excised root segments. In excised root

tips, there appeared to be weak K^+ pumps at both sites in the root.

This study also showed that ^{86}Rb can only be a suitable tracer for K^+ ions as far as transport into the symplast and into the xylem were concerned. There was a discrimination against Rb^+ ions at the tonoplast. It was found that accumulation of K^+ ions in the vacuole was about 1.8 times greater if ^{42}K was used as a tracer than if ^{86}Rb was used.

In an attempt to distinguish the effect of changes in the oxygen state of the root medium on transmembrane potentials, it was found that cortical cells depolarized from -132 mV to -122 mV under anaerobic conditions. Moreover, there was a transient hyperpolarization shortly before the depolarization took place, which was similar to the effect of CN^- on membrane potentials found by other workers. When DNP was used as an inhibitor, cells depolarized by about 70 mV. This potential change is accounted for by an electrogenic ion pump component. When roots were excised, cortical cell depolarized. This was followed by a hyperpolarization of cells and accompanied with the loss of K^+ ions from the excised tissue.

No enhancement of uptake of K^+ was observed in young roots adapted to anaerobic conditions that were transferred to aerobic conditions. The observations by other workers of enhanced uptake in mature plants was accounted for through the adaptation of a greater secondary root sysytem.

DECLARATION

I certify that this thesis does not contain any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Pitul Aramsattana.

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Abbreviations and Symbols

AA, AN	aerobic and anaerobic tissue labelling for aerobically grown plants
ABA	abscisic acid
C_c, C_v, C_x	ion concentration in the cytoplasm, the vacuole and the xylem
DNP	2,4-Dinitrophenol
F.Wt	fresh weight of root tissues
k_s, k_L	the short-term and long-term rate constant of isotope exchange, respectively
E_p, E_t, E_x	electrical potential across the plasmalemma, the tonoplast and the xylem, respectively
NA, NN	aerobic and anaerobic tissue labelling for anaerobically grown plants
PD	electrical potential difference across cell membranes
Q_c, Q_v, Q_x	ion content in the cytoplasm, the vacuole and the xylem, respectively
Q	the total content of ions in tissues
S_o, S_c, S_v, S_x	the specific activity of the labelled solution, in the symplast, the vacuole and the xylem vessels
T	tissue loading time in a labelled solution
V_c, V_v	the cytoplasmic and vacuolar volume

Y	the total amount of tracer which leaves the tissue at the end of washout
Y_r	the amount of tracer in the labelled tissue at the end of washout
Y'	the total loss of tracer from a portion of an intact root into the washout medium
Y''	the total loss of tracer from a portion of an intact root into the xylem vessels
Y_{Fe}'	the loss of tracer from the free space and the short-term component into the root medium from a portion of an intact root
Y_e'	the short-term loss of tracer into the medium from a portion of an intact root
Y_e''	the short-term loss of tracer into the xylem from a portion of an intact root
Y_s, Y_L	the short-term and long-term component of tracer in root segments at the end of loading
Y_{in}, Y_x, Y_t	the amount of tracer in the labelled portion of an intact root, in the upper part of the plant via the xylem vessels and in the root tip region at the end of tissue loading
Y_{up}, Y_{down}	the amount of tracer accumulated in the tissue above and below a labelled portion of an intact root, respectively, at the end of tissue loading

ϕ_{in}	the apparent influx of ions into the labelled root tissue
ϕ_v, ϕ_x	the net flux of ions into root vacuoles and the xylem vessels, respectively
ϕ_{oc}, ϕ_{co}	ion flux from and into the outer space and the cytoplasm
ϕ_{cv}, ϕ_{vc}	ion flux from and into the cytoplasm and the vacuole
ϕ_{cx}, ϕ_{xc}	ion flux from and into the cytoplasmic symplast(c) and the xylem
ϕ_{ox}	an apoplastic flux from the medium into the xylem

Chapter 1

Introduction

The roots of plants have several functions. They act as water and nutrient absorbing organs. They provide anchorage to the plants, making it possible for the shoots to stand upright. Some growth regulators and hormones are synthesised in the roots and in some species they provide for food storage. In their role of absorbing organ, the roots take up both organic and inorganic substances and transport them to the shoots via the xylem vessels. Photosynthesis products of the leaves, such as carbohydrates, are translocated to the lower part of the plants, and finally to the roots, along the phloem. The inter-dependence between the shoot and the root makes it difficult to study the function of one without involving the other. Examples of these are the reduction of shoot growth due to the failure in the production of cytokinins and gibberellins of the root (see Kramer, 1983), and the increase in ion uptake into roots with increased shoot illumination (Hatrick and Bowling 1973). When the root absorbs nutrients (mostly inorganic) from the surrounding solution or soil, some are accumulated by the root cells and some are transported to the upper part of the plant via the xylem vessels.

Following Pitman(1971), the term "uptake" will be used to include both accumulation and transport. The transport up to the shoot is driven partly by water flow to replace that lost by evaporation, and partly by active absorption processes in the root cells (or at the boundary with the stele). Since ions move along with water molecules, changes in the rate of transpiration affect ion uptake. Movements of ions in the root occur not only in the upward direction. Re-translocation of ions does take place in the phloem down to the root, particularly to the tip region, if the supply to the shoot is in excess (Pitman 1972b). In young plants when the seeds are the main source of nutrients, downward transport via the phloem can be substantial. However, this becomes less significant with plant age when food reserves in the seeds become deficient and the roots become the primary source of inorganic nutrients for the plants. Therefore, consideration of inorganic transport is usually confined to the xylem conveyer,

although it needs to be demonstrated particularly in young seedlings that ion transport via the phloem is relatively negligible.

The environment in which roots are grown seems to be another important aspect for ion uptake into roots. There is considerable evidence suggesting that roots grown under low external concentration are more efficient in taking up ions than those grown in a relatively greater concentration (Cram and Laties 1971, Leonard and Hanson 1972 , Leigh and Wyn Jones 1973, and Pettersson 1975). In addition, some scientists have suggested dual mechanisms of ion uptake (see Epstein 1976 and references therein); one of which operates under low external concentrations (< 1 mM) and the other under high concentration (> 1 mM). These are known as mechanism I and II, respectively. In a slightly different fashion, others (Glass 1976a, and Glass and Dunlop 1978) proposed a so-called allosteric regulation in which the uptake depends on the salt status of the roots, and is greater if the internal content is small. Work relating the intercellular content to the uptake mechanisms is available from Nissen (1974 and 1980), Pettersson and Jensén (1978 and 1979) and Pettersson (1981) who suggested that the salt uptake is governed by a negative feedback from the concentration in the cytoplasm. However, a more recent work on the relation of uptake and transport to the internal concentrations has been reported on the evidence of non-allosteric regulation (Drew and Saker 1984). Instead, the uptake and transport were suggested to be regulated by ion flux into the xylem.

1.1 Pathways of Ions Across The Roots

To traverse the root, ions move along two pathways, the symplast and the apoplast. The former commences when ions are absorbed into cells and transported in the cytoplasmic continuum through plasmodesmata channels into the stele. The latter is the diffusion of ions through intercellular space, which also includes the intermicellar and the interfibrillar spaces of the cell wall.

During the development of the endodermis, a

suberin-like material deposits in the tangential direction between the adjacent cell walls. This occurs at the primary stage of the development and fully expands, covering the whole cell length and forming a so called "Casparian band" at the tertiary stage. Whether the presence of suberin could effectively block the apoplastic transport across the endodermis is still uncertain. In earlier investigations (Robards et al. 1973, Tanton and Crowdy 1972, and Robards and Robb 1974) using electron-dense metal ions, it was found that the ions could travel as far as the endodermal cells. This evidence suggested that suberin material could completely block the apoplastic transport at the later state. As a consequence, solutes can only enter the endodermis via the symplast. This idea has been supported by many authors, such as Läuchli (1976), Anderson (1976), Bowling (1976), Spanswick (1976), Pitman (1977), and Lüttge and Higinbotham (1979). In conflict with the above, a possibility for apoplastic transport into the xylem at the tertiary state of endodermal development was proposed by a number of authors (Clarkson and Sanderson 1971, Clarkson 1979, Stephens and Clarkson 1981, and Sanderson 1981 and 1983). There was evidence in maize roots (Stephens and Clarkson 1981) showing that water uptake was not affected greatly by the deposit of suberin at the endodermis and hypodermis. In barley (Sanderson 1983), water uptake and Ca^{+2} transport across the tertiary state endodermis were at a measurable amount. In onion roots, it was found that suberised wall was permeable to water and suggested that it was microporous (Clarkson et al. 1978). Before any conclusion can be made, more investigations concerning the effect of suberin on ion permeability are required.

On the absorption site of ions into roots, it was suggested that epidermal and cortical cells played the major role, particularly when the external concentration was small (see Anderson 1976, Lüttge and Higinbotham, 1979). Under the above condition, a direct absorption from the apoplast into the cortex was possible. Nevertheless, some experimental evidence by a number of scientists (see Kochian and Lucas 1983, Vakhimistrov 1967, Bowling 1976, Van Iran and Boers-Van der Sluijs 1980a) suggests that the epidermal cells can absorb ions more readily

than cortical cells, being in direct contact with the medium. When ions enter the symplast, they are transported from cell to cell via plasmodesmata and cytoplasmic streaming circulates them within the cell. Investigation of roles of the streaming in symplastic transport suggested that the streaming hardly affected the transport (Glass and Perley 1979). Plasmodesmata, on the other hand, might facilitate the transport to some extent particularly in the stele where a greater number was found than in the cortex (Clarkson et al. 1971, Helder and Boerma 1969). Moreover, the finding of a greater number of plasmodesmata in hair cells than in hairless cells (Vakhmistrov et al. 1972) seems to indicate the active zone of ion absorption into roots.

Movement of ions along the symplast commences when they are absorbed across the plasmalemma. After they enter the cells, exchange of ions across the tonoplast occurs together with movement toward the xylem. Since a plant root consists of several types of cells with a range of different properties, there have been difficulties in revealing a clear picture of root transport mechanism. Ion uptake also depends on the root environment, the preferences of the various plant species for particular ions, and on the salt status of the roots. However, it is generally found that the salt concentration in root cells is much greater than that in the surrounding medium. This leads to an expectation that the absorption of salts into roots involves an active process, although a greater internal concentration does not itself imply active transport. The status of an ion species depends on its electrochemical potential so that knowledge of transmembrane potential is essential in any consideration of the uptake process. Much experimental evidence shows that an active process is involved in the uptake of many ion species into plant roots.

The term "active" is referred to as a mechanism by which an expenditure of metabolic energy is required, and occurs when ions are transported against the electrochemical gradient. If ions move under the action of electrochemical forces, the transport is referred to as "passive". Besides the transmembrane potentials, concentrations of the ion species on both sides of the membrane, and knowledge of ion fluxes across

the membranes are required.

Previously, the studies of the uptake mechanism have been conducted on excised root tips or root segments. These studies are reviewed in the following sections. The main purpose of this thesis is to describe ways of studying ion fluxes in a portion of an intact root, since this should give information about the properties of root cells under normal growing conditions. The methods developed in chapter 2 are applicable both to intact and excised roots. It is therefore desirable to make a comparison study of roots in both intact and excised states, to indicate the effect of excision on these cellular poperties and to provide a comparison with the results of other workers.

1.2 A review of compartmental flux studies

Ion fluxes in plant cells and tissues have been studied by using radioisotopes as tracers for the studied ions. The general method for this study is by loading the tissues in a labelled solution for a period of time and washing out with a non-labelled solution at several time intervals. Time course of the tracer content in the tissue during washing is obtained by adding the amount of tracer washed out into the medium to the amount of tracer found in the tissues at the end of the experiment. A semi-logarithmic plot of these values, generally, shows three exponential terms. The time constants of these terms are related to tracer equilibration in the free space, the cytoplasm and the vacuole. These are used in mathematical procedure to estimate ion fluxes across cell membranes; the plasmalemma and the tonoplast, and the internal content in the cytoplasm and the vacuole. The model developed from this study is known as a three compartmental model. The method of study as described above is adopted from the study in large algal cells, *Nitellopsis obtusa*, by McRobbie and Dainty (1958).

Kinetics of ion movement in higher plant cells have been studied in storage tissue of red beet (Pitman 1963, 1964, Poole 1969, 1971) and carrot (Cram 1968a and b), root of barley (Pitman and Saddler 1967, Pitman 1969), corn (Torii and Latties 1966, Cram 1973), broad bean (Pallaghy and Scott 1969), onion (Macklon 1975a and b) and pea (Etherton, 1967, Higinbotham et al. 1967), coleoptiles of oat (Pierce and Higinbotham 1970) and epicotyls of pea (Macklon and Higinbotham 1970). In these studies, the whole segment of tissues was treated as a combination of uniform cells.

To estimate ion fluxes, mathematical analysis derived for steady state conditions of tissues was commonly used. Pallaghy and Scott (1969) showed that exact solutions for fluxes across compartmental boundaries (i,e the plasmalemma and the tonoplast) were possible, provided that there was no net flux across them and the partial fluxes were not functions of time. They also considered cases when there was a net flux into the inner cell compartment, and proposed approximate solutions for

ion fluxes and compartmental contents in terms of observable quantities. However, the validity of their solutions when transport to the stele was included was questioned by Walker and Pitman(1976).

In the case of root tissues, there are some difficulties due to the fact that they do not simply act as an ion accumulation organ. As pointed out by Pitman (1971), the root is an organ which secretes inorganic substances to the xylem for transport to the shoot. This provides complexities in the pathways of ion movement. In this case, the exudate through the root cut end would mix into the medium and, consequently, the efflux across the plasmalemma would be overestimated. Based on the finding in barley roots that the amount of Cl^- loss through the cut end was much greater than that across the cell surface, Pitman (1971) proposed a model for ion transport (see Fig. 2.1 in chapter 2) which included fluxes across the xylem and apoplastic transport into the xylem. However, the latter and the efflux from the xylem were suggested to be negligible. Pitman model (1971) and the method for flux estimations were followed by many authors subsequently for Na^+, K^+ and Cl^- ions (Jeschke 1972, 1973, 1977 and 1982, Davis and Higinbotham 1976 and Behl and Jeschke, 1982).

The validity of negligible xylem efflux as in the above studies has been questioned by Johanson and Cheeseman (1983) and Johanson et al. (1983). According to their experimental results, a considerable amount of Na^+ ions in the xylem were re-absorbed into the cortex of corn mesocotyl and an active efflux from the xylem was suggested. The finding of re-absorption ability of cells surrounding the xylem vessels has been mentioned elsewhere (Jacoby, 1965 and Shone et al., 1969). If this is correct, the influx to the vacuole and the efflux from the cytoplasm to the medium will be underestimated to some extent.

As memtioned earlier, the tertiary state of endodermal cell development is used as an indication for a blockage of the apoplastic transport (Anderson 1976). In the case of barley roots (Pitman 1971, Jeschke, 1972, 1973, 1977 and 1982 and Behl and Jeschke 1982), a support for this is from Danilova (1981) who found that the development was complete at the

distance of only about 6-7 mm from the root tip. The endodermis of corn roots, however, does not reach this stage until at the distance of about 20-30 mm behind the tip (Peterson and Perumalla, 1984). The assumption used by Davis and Higinbotham (1976) is, therefore, questionable. In addition, if the suberin like substance which forms the Casparian band is permeable to water (Clarkson et al. 1978), there is no reason why ions cannot permeate the substance, since they are transported together with water molecules.

Another important point of view which should be raised is that most of the above work which was performed by taking the xylem transport into account dealt with excised tip segments. As is known, cells in this region are non-homogeneous and ion fluxes are at best an average of measurments of many cell types. According to this and to avoid complications from the presence of the stele, work in the past by Pallaghy and Scott (1969) and Cram (1968a and b and 1973) were performed in isolated cortices of mature roots and storage tissues, respectively.

The results of the ionic flux measurements mentioned above fall into two categories; one described the movements of ions in low salt roots and the other in high salt, or "salt saturated", roots. Following Pitman (1976), low salt roots represent those which were grown in a weak $CaSO_4$ solution and the studied ions were introduced into the medium for a period of time prior to the experiments. On the other hand, high salt roots represent those grown in a complete nutrient solution throughout their life. The results of measured fluxes in low salt roots generally are much greater in magnitde than those in high salt roots. This seems to confirm the importance of salt status in the root on ion uptake, as reported by Glass and Dunlop (1978) and Petterson (1981). In addition, a greater ion loss through the cut end than across the root surface was often found in low salt barley roots (Pitman 1971, Behl and Jeschke 1982), while the reverse was true in high salt corn roots (Davis and Higinbotham 1976). Before one can be certain of whether this smaller loss of ions through the cut end is a general characteristic of high-salt roots or the behaviour of the plant species, more experiments for high salt roots in other plant species should be carried out. So

far, only work by Davis and Higinbotham (1976) and Pallaghy and Scott (1969) described ion movements in high salt roots. Note that the former have taken transport across the xylem into account, while the latter have performed in isolated cortices.

It is interesting to point out that all of the above work concerning efflux measurement were made by utilising the three compartmental model. This assumes that ions are taken up from the free space into the cytoplasm before entering the vacuole. Another group of scientists have proposed that there may be a fourth compartment residing in the cytoplasm (Pallaghy et al. 1970, Lüttge and Pallaghy 1972), or at the plasmalemma (Bange, 1977). This idea was brought about by the finding that at low external concentrations, irregularity of ion efflux from corn root segments was observed (Pallaghy et al., 1970), and ion accumulation in the vacuole occurred after a lag phase of transport into shoots (Bange, 1979). However, the irregularity was suggested to be due to the exchange between nonhomogeneous cells when excised barley roots were used (Behl and Jeschke, 1982). In later studies when intact roots of wheat (Erlandsson, 1979) and cucumber (Jensen and Kylin, 1980) were used, the cause of the irregularity was suggested to be the response of changes in external salt concentration. In more recent studies in intact sunflower roots by Jeschke and Jambor (1981), the irregularity of Na^+ efflux was found after an addition of K^+ ions into the root medium and was suggested to be due to some deviation from the steady state conditions.

It is known that root excision disrupts the supply of ions from the seed, in the case of young seedlings, and some energy supply from the shoot. There have been several reports on the inter-dependence between the root and the shoot by many workers (Pitman 1965a, b and 1966, Hatrick and Bowling 1973, Pitman and Cram 1973, Graham and Bowling 1977, Bowling and Dunlop 1978 and Jeschke 1982). Examples of these are on the increase uptake after an increase in shoot transpiration and on the reduction of ion uptake after the shoot has been removed. Any changes which affect ion uptake will cause corresponding changes in the fluxes at the root cells. To solve the problem of the shortage of food supply after root excision, some workers,

such as Macklon (1975a) and Behl and Jeschke (1982), added sucrose to the root medium. The latter reported that such treatment did not solve the decline of ion efflux. It is, therefore, open to question whether the results from excised roots can be used to explain the mechanism of ion uptake into intact roots.

Measurements of ion fluxes in intact roots were made first by Jeffries (1973) and followed by Jeschke and Jambor (1981). As done in large algal cells or in excised roots, these workers treated the whole root system as a group of uniform cells and the former did not take xylem transport into account. This method can be argued in view that steady state conditions of the roots cannot be met, particularly where the rapid growth of cells in the meristematic region is taking place. Their observations are, at best, an average over a wide range of cell types. In fact, this problem was recognized by Pitman (1963) in his early studies of higher plant roots, but despite this no attempts have been made to treat in isolation a more homogeneous portion of an intact root. Jeschke (1982) attempted to avoid some of the problems of inhomogenuity by removing the tip, although he recognised that this causes some damage to the plant system. The effect of injury from root excision on ion uptake and fluxes were reported earlier in maize (Jackson et al., 1973, and Gronewald and Hanson, 1980) and barley (Pitman et al., 1974a). Morover, caryopsis was also removed to minimise the downward transport of ions from the seed. Further studies made by Jeschke et al. (1983) showed that in contrast to intact plants for which the K^+ content of the roots continue to increase with time, the roots of plants whose caryopsis have been removed rise and then fall in a similar manner to excised roots. On the basis of vacuolar Na^+/K^+ exchange, they suggested a possibility of phloem transport of K^+ to the region containing young cells. This clearly indicates that if whole roots of plants are to be investigated, the model for ion transport has to be developed and the phloem fluxes must be taken into account.

As being noted, most of the previous work on flux measurements in plant roots were performed in low salt ones. An

advantage of using low salt roots is that the suger level in the tissues is greater than in high salt ones (see Pitman 1976 and 1982). Low salt roots would have energy to continue their activities longer than high salt roots, after being isolated from the main plants. This could be one reason for the choice of low salt roots for flux measurements in most experiments concerning with excised roots. When these roots are exposed to a particular ion during the loading stage, this has the advantage of a constant specific activity throughout the plant. It is assumed by many authors that the specific activity of the cytoplasm and the xylem are the same as that of the medium (Pitman 1971, Jeschke, 1973, 1977 and 1982).

A disadvantage in using low salt roots is that the condition of equal specific activities between the cell interior and the label medium during isotope loading may only be approximated in practice. Davis and Higinbotham (1976) showed that the specific activities of the symplast and the xylem of high salt roots were much smaller than that of the medium in tracer experiments. Since the medium does not contain the ion to be studied in the tracer experiments, ion fluxes into the roots during the loading are likely to change rapidly with time. This possibility is not covered in the simple mathematical model of a root system which has been used in flux analysis. This model has been developed with the assumption that the fluxes do not change with time. Moreover, when intact roots were used (Jeschke and Jambor, 1981), tissue loading was made over a period of 24 hrs. This would allow root elongation to occur and the roots are far from being in a steady state. Therefore, roots studied in these conditions do not necessarily give any information about the roots grown in complete nutrient solution.

Experimental evidence supporting the view that transport mechanisms may differ according to salt status of the root has been obtained. It was found that Cl^- ions were transported actively into the xylem of low salt roots (Pitman 1971). This was not the case when high salt roots were used (Davis and Higinbotham 1976). It should be mentioned that the conclusion made by Pitman (1971) was based on finding a much greater Cl^- loss through the cut end than through the root

surface which was confirmed by the use of an inhibitor (Cram and Pitman 1972). Side effects from the use of an inhibitor was later discussed by Lüttge and Higinbotham (1979).

Due to the difference in sugar level between low salt and high salt roots, there is reason for believing that electrogenic pumps disappear after root excision, and that they do so sooner in normally grown plants than low salt ones. However, after about 10 hours of washout, a rapid fall of ion content in low salt excised roots was observed although some sucrose was added to the root medium (Behl and Jeschke 1982). It was suggested that this is due to a shortage of energy reserves. This problem and the ones of the rapid change in ion fluxes during tissue loading when low salt roots are used, and on the complexity caused by non-homogeneous cells at the tip region, may be solved if the studies are made in a mature portion of intact high salt roots.

In order to test whether the fluxes are purely passive or involve active transport, it is necessary to use the Ussing-Teorell (flux-ratio) equation. This relates ion fluxes to electrical potential difference across the membrane

$$\frac{\phi_{io}}{\phi_{oi}} = \frac{C_i}{C_o}\left[\exp(Z_j FE/RT)\right] \qquad (1.1)$$

where ϕ_{io} and ϕ_{oi} represents passive ion fluxes from and to the inside (i) and outside (o) of a cell membrane. The measured value of the flux ratio can be compared with the predicted one, providing that the electrical potential across the membrane and the concentrations inside and outside the membrane are known. If the measured ratio differs significantly from the calculated passive flux ratio, active transport is inferred and the active component of the flux can be determined.

After the model of ion transport was proposed by Pitman (1971), only Davis and Higinbotham (1976) appear to have utilised the above relationship. According to these authors, the efflux from the xylem could not be determined hence, only the predicted value was obtained assuming equal concentration between the cortical and stelar parenchyma cells. They concluded that K^+

ions are transported actively into the symplast and the xylem vessels, while Cl^- diffuses passively into the xylem, after being actively transported across the plasmalemma. The above conclusion on the K^+ pump at the plasmalemma is in contradiction with the result obtained from uniform cortical cells made by Pallaghy and Scott (1969). The result on the Cl^- pump is inconsistent with the result obtained from low salt barley roots (Pitman 1971). If it is not due to plant species, the contradiction results on the K^+ pump can be attributed to the difference in functions between cells in the tip region and in the mature region. Young cells at the tip are more active in accumulating salts from the medium. The passive Cl^- transport into the xylem could be due to the higher salt status of corn roots than barley roots.

The prediction of an electrogenic pump across the stele disagrees with the "symplasm theory" proposed by Craft and Broyer in 1938. Originally, the idea of passive movement of ions into the xylem was based on the assumption that oxygen concentration in the inner cell layers were too small to account for active transport. On investigation of the respiration rate, the result obtained from isolated stele (Laties and Budd, 1964 and Hall et al., 1971) disagreed with that from root segments (Yu and Kramer, 1967). A more simple approach which has been widely used is to investigate the effect of the uncoupler Carbonyl cyanide m-chlorophenylhydrazone (CCCP), phytohormone Abscisic acid (ABA), or protein inhibitors cyclohexamide (CHM), amino acid analog p-fluorophenylalanine (p-FPA) and 2,4 dinitrophenol (DNP) on xylem exudate (Läuchli et al. 1971, Cram and Pitman 1972, Pitman et al. 1974a, Wildes et al. 1976, Behl and Jeschke 1981 and Kuiper and Boer 1980) and the effect of p-FPA on xylem potential (Dunlop, 1982). These workers suggest the existence of pumps for the delivery of ions into the xylem. A review of ion transport into the xylem by Pitman (1977) provided some experimental evidence on this matter. Although Pitman and his associates were able to show that the reduction of the exudate occurred about 2 hrs after the reduction of the influx at the outer cell membranes, the simultaneous increase in vacuolar influx and ATP level lead to an ambiguity in the results. Side effects from the use of xylem inhibitors, together with the dependence of the

results on envirnmental conditions and endogeneous hormones are discussed by Lüttge and Higinbotham (1979) and Pitman (1977). The same side effect could explain the depolarization of cells when xylem potentials for intact roots were measured under a p-FPA containing solution by Dunlop (1982).

It should be mentioned that although an inhibition effect on the xylem exudate was found by the above authors a stimulation effect was also reported by a number of wokers, particularly when ABA was used. Examples of ABA-induced exudation in other plant species can be found elsewhere (Glinka 1973, 1977 and 1980, Collins and Kerrigan 1974 and Karmoker and Van Steveninck 1978).

Due to the ambiguity in the above result for active inward ion pumps into the xylem, a method to measure the xylem efflux should be developed so that one can use the Ussing-Teorell equation to predict the transport mechanism. To do so, knowledge of trans-membrane and trans-root potentials is essential. This is reviewed in the following section.

From the above review, some further aspects needing consideration are.

(1) If the uptake into the root is, partly, governed by the shoot, will the difference in ion net flux into intact roots upset the predicted existence or non-existence of ionic pumps?. Measurements on intact roots by Jeschke and Jambor (1981) cannot be related to the flux-ratio equation, since membrane potentials were not measured. As far as transport at the cortical cells is concerned, Pitman et al. (1976) point out that it is simpler to use excised roots. However, there is no evidence that the mechanisms of transport in excised roots are the same as those in intact roots.

(2) In efflux experiments , Behl and Jeschke (1982) found a difficulty when the root tip was included, due to longitudinal transport of ion between meristematic and differentiated cells. By discarding the tip, Jeschke et al. (1983) introduced another problem due to root damage. Davis and Higinbotham (1976) mentioned that errors in flux measurements could occur when using excised tip segments. It is possible that

the contradictory results between corn roots and broad bean roots on the K^+ inward pump at the plasmalemma are due to non-uniform fluxes in excised root tips.

(3) Most fluxes in plant roots, so far, have been measured using low salt roots with the tissue loading over a long period of time so that a steady state of fluxes can be achieved. This method can be criticised since the fluxes are not constant during the loading period. Thus, the use of an analytical procedure assuming steady state conditions may be questioned. Besides, the elongation of intact roots can bring another complication to the studied system. Mertz and Higinbotham (1976) mentioned some difficulties in measuring electrical potentials in low salt roots due to a low tugor pressure of the cells. This causes a tough cell wall and, subsequently, affect the potential values (Anderson and Hinginbotham, 1975). These problems seem to require more studies in high salt, intact roots with as much uniformity as possible in the cells.

To deal with the above problems, it is necessary to develop a method of measuring fluxes in intact roots. As suggested by Pitman (1976 and 1982) and by the evidence in corn roots (Davis and Higinbotham, 1976), one cannot assume the same specific activity of the symplast as the medium if high salt roots are used. To study the mechanism of ion transport across the stele, utilisation of the flux-ratio equation which requires the knowledge of xylem (fluxes and xylem potential) seems to be a better method than use of plant hormones.

1.3 A Review of Transmembrane Potential in Higher Plant Roots

It is common practice to measure transmembrane potentials by inserting microprobes filled with high salt concentration solution into plant cells. The size of microelectrode tips is not greater than 1 micrometer in diameter. The potentials are measured relative to the external medium and are almost always negative. The magnitude varies from plant to plant and depends on the concentration of the bathing solution.

Unlike the wall of animal cells, those of plant cells are composed of cellulose which makes it hard to insert a fine microelectrode. Higher plant roots consist of layers of cells, and each cell has two membranes; the plasmalemma and the tonoplast which enclose the cytoplasm and the vacuole respectively. The vacuole normally occupies 85-90% of the cell volume and the cytoplasm is about 1 micrometer thick (Higinbotham, 1970). Potentials measured by this means are likely to be those of the vacuole. A number of workers attempted to measure the potential across the tonoplast. Some suggested no potential difference (Etherton and Higinbotham 1960, Etherton 1970, Greenham 1966, Nobel and Craig 1971), while others suggested that the potential in the vacuole was positive in relation to that of the cytoplasm, but small in magnitude (Dunlop 1976, Glass and Dunlop 1979, Davis 1972). However, in some cases the vacuolar potentials were as much as 35-40 millivolts more negative than the medium (Ginsburg and Ginzburg 1974, Mertz and Higinbotham, 1976).

To avoid the problem of the tough cell wall and uncertainty in the electrode tip location, some workers have measured the potential of isolated vacuoles (Wagner and Siegelman 1975, Doll and Hauer 1981 and Miller et al. 1984). The result was between -60 mV and -70 mV, relative to the medium.

When whole roots are used, the result of potential measurements across the tonoplast depends very much on the technique and the conditions of the plant roots. It was suggested (Mertz and Higinbotham, 1976) that if root cells lost their turgor pressure, the plasmalemma would be pushed in and the

tip would reside in the vacuole after puncturing the cell. When a greater value than a few millivolts was found, it was suggested (Dunlop, 1976) that the potential is due to leakage along the wall of microelectrodes across the plasmalemma leading to a decline in the potential when the tip resides in the vacuole.

In a few cases potentials across the roots were measured by advancing the microelectrodes inward, from the epidermal cells to the stele (Dunlop and Bowling 1971b, Bowling 1972, Dunlop 1973, 1976 and 1982, and Ginsburg and Ginzburg 1974). This method was criticized by Anderson and Higinbotham (1975) (however, see Dunlop 1976), since there could be leakage along a trail of ruptured cells and also blockage of microelectrode tips by cell debris after several impalements from cell to cell. In the case when the microelectrode tip could not be observed, the penetration of the tip into a cell was determined by an abrupt change of the potential (Dunlop, 1976). Some workers found potential gradients between the epidermis and the cortex when microelectrodes were advanced radially (Davis 1972, Mertz and Higinbotham 1974 and 1976). However, others reported contradictory results when excised roots were used (Dunlop 1976, Dunlop and Bowling, 1971a). There was no difference between the stelar and cortical potentials (Bowling 1972, Davis 1972 and Dunlop 1976). The latter was also true when *Avena* coleoptiles (Higinbotham et al., 1964) and pea epicotyls (Macklon and Higinbotham, 1968) were used. The potential in hair cells was often found to be the same as that of epidermal cells (Etherton and Higinbotham 1960, Mertz and Higinbotham, 1976). Along the longitudinal direction of the roots between 2 mm and 20 mm from the tip of broad bean roots (Scott et al., 1968) and between 5 mm and 40 mm of barley roots (Mertz and Higinbotham, 1976), no potential gradient was found.

When excised roots were used, it was found that the magnitude of potential was initially small and gradually became greater with ageing (Macklon and Higinbotham, 1968, Pitman et al. 1971, Lin and Hanson 1974b, Glass and Dunlop 1974). Time taken for the potentials to stabilise was between 5-6 hrs after ageing. When cells at the cut surface were measured, it was found that the potential was much smaller in magnitude compared to

cells away from the surface (Mertz and Higinbotham, 1976). There have been several explanations for depolarization of the cells after cutting, such as an increase in cytoplasmic salt concentration (Pitman et al., 1971), mechanical stress (Novacky and Jones, 1974), cutting injury (Mertz and Higinbotham, 1976), short circuiting through plasmodesmata (Anderson and Higinbotham 1975), and a decline of an electrogenic component of cell potentials (Lin and Hanson, 1974).

For the measurement of xylem potential, it is difficult to advance microelectrodes into the inner layers of root cells. Apart from the possibility of short circuiting, the tip of the electrode could be broken after penetrating through several cells, resulting in the observation of a less negative potential. Furthermore, it is difficult to observe the location of the electrode tip in inner cell layers. Only in a few cases, such as white clover roots (Dunlop, 1982), could the tip be observed. To solve the above problems, another method employed is to cut the roots and measure the potential of the exudate in relation to the medium (Shone 1969, Davis and Higinbotham 1969, Dunlop and Bowling 1971b and c). When root segments were used, it was suggested (Davis and Higinbotham, 1969) that low potentials could be due to the presence of short-circuiting pathway. In general, it is found that the potential is smaller in the inner cell layers. It ranges from 120 mV or more in the cortex to about 30-35 mV in the xylem. In the case where intact roots were used (Dunlop, 1982), the xylem potential was as large as -70 mV. rel. external medium

With a knowledge of transmembrane potentials, it is possible to test whether the movement of an ion species is due to an ionic pump with the knowledge of transmembrane potentials, providing that the concentration in the cell interior is known. This is based on the assumption that ions move passively and independently across a membrane under an electrochemical driving force. At equilibrium when there is no net flux, the potential is equal to the Nernst potential. That is

$$E = E_i - E_o = \frac{RT}{Z_j F} \ln \frac{C_i}{C_o} \qquad (1.2)$$

where E is the electropotential difference between the inside (i) and the outside (o) of the membrane; R, the gas constant; T, the absolute temperature; Z_j, the valence of the ion species; F, the Faraday constant; and C, the concentration of the ion inside (i) and outside (o). If the measured value does not match with the predicted one, the existence of an electrogenic pump is suspected. It is also possible to deal with cases in which the ions are not moving independently. In living systems, movements of ions are more complicated, since they are present in pairs; cations and anions, and it is not possible to consider the movements of one species without including the others. Under these circumstances, equilibrium might not be reached. Ions which are studied most in plant tissues are K^+, Na^+, and Cl^-. If the electropotential gradient across the membrane is linear and the ions are moving passively and independently across a membrane, at electroneutrality the potential can be determined by the Goldman equation as

$$E = \frac{RT}{Z_j F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o} \qquad (1.3)$$

where P is the permeability coefficient , and the subscripts o and i represent the external and internal concentration of the ion. To determine the coefficients, potential measurements are made with a range of external concentrations. Examples of the use of the above methods are provided by Etherton and Higinbotham (1960), Higinbotham (1970), Higinbotham et al. (1970), and others (see reviews by Higinbotham 1973, Higinbotham and Anderson 1974 and Spanswick 1981). In general, it was found that anions were pumped inward, while Na^+ was pumped outward. Transport of K^+ was not conclusive, despite being the most concentrated ion. One of the proposed reasons for passive movements of K^+ was related to the fact that the membrane was more permeable to K^+ than others

and much energy would need to be expended to maintain it away from its equilibrium state. it was suggested that if its transport is electrogenic, then it occurs in an outward direction (Higinbotham and Anderson, 1974). Other possibilities are that K^+ ions may be co-transported with Cl^- ions (Bowling, 1966), transported inward as one to one exchange with H^+ ions or co-transported with HNO_3 ions at the plasmalemma (Poole, 1966).

The existence of ionic pumps was confirmed by using respiratory inhibitors, such as cyanine (CN^-), azide (N_3^-), 2,4 dinitrophenol (DNP), or carbon-monoxide saturated solution (Davis and Higinbotham, 1969, Higinbotham et al. 1970, Anderson et al. 1974, and Drake 1979) to halt metabolically driven electrogenic pumps. When the solution containing an inhibitor was introduced into the root medium, a rapid cell depolarization occurred and the reverse was true when the solution was withdrawn. Since the measured potential during depolarization of the cells was close to the predicted value, it was concluded that this potential was derived from passive diffusion alone. When carbon monoxide was used (Anderson et al., 1974), depolarization occurred only in the dark. The effect of light on membrane potentials was well evidenced in aquatic higher plants (Spanswick 1973) and in *Riccia fluitans* (Felle and Bentrup, 1976). Further developments to separate the passive and active components are provided by Higinbotham and Anderson (1974) and Cheeseman and Hanson (1979a and b). The application of the theory is described by Anderson et al. (1977), and reviewed by Spanswick (1981).

1.4 A Short Review of Research in Rice Seedlings

Rice is one of the most widely cultivated crops. In general, it is classified into 2 groups; upland rice which can grow in dry areas several metres above the sea level, and lowland rice which grows well in water-logged fields. Due to this adaptive ability, rice growing can be seen in area from latitude 53° N in China, on the equator, to 35° S in Australia. However, the tropics and temperate regions seem to be the best locations, for plenty of water helps to improve the yield. In South-East Asian countries rice seedlings are transplanted in a flooded field. Plants at that stage are about 20-30 days old, having initially grown in a nursery bed under drier conditions. Water-logging is necessary for the plants until about 20 days before harvesting. This method of cultivation seems to be unique in comparison to other crops.

The fact that rice grows well under anaerobic conditions has interested scientists, since the beginning of this century. Between 1913-1916, Harrison and Aiyer investigated the composition of gases that escaped from rice fields and found that they consisted of methane and nitrogen. They suggested that oxygen molecules developed at the soil surface and moved downward to the deeper soil layers due to shoot transpiration. This idea was well accepted from that time until the 1940's when Van Raalte (1940 and 1944) analysed the oxygen content in the root cortex and found that it did not depend on the content of the culture solution. Meanwhile, Vlamis and Davis (1943 and 1944) demonstrated that rice could also grow well in solutions in which air was replaced by nitrogen, and salt uptake into roots was as high as those grown in aerated solution. Based on these results, it was suggested that the supply of oxygen to the roots was from the shoots. This finding was confirmed later by a number of workers (Katayama 1961, Barber et al. 1962, Armstrong 1971 and Webb and Armstrong 1983). According to Yoshida (1981) air spaces in the cortex also develope in upland rice roots but to a lesser extent.

On morphology and growth, it was found that branched roots developed during maturity and served to supply

oxygen for roots penetrating deeper into the soil (Alberda 1954) and they developed sooner if the roots were subject to anoxia (John et al. 1974). Under such circumstances, the plants were more effective in transporting oxygen to the oxygen-deficient area of roots than those formed under aerobic conditions (Valoras and Letey, 1966). In comparison to many plant species, rice could germinate under strict anoxia (Vartapetian et al., 1978) and even in a vacuum (Opic 1973), but root growth was inhibited in both cases. In four week old plants (John et al., 1974), root dry weight was reduced by 25% after being subjected to anoxia.

A large number of investigations on rice have been made during the last decade. Some were aimed at protein synthesis and energy levels in the embryo and coleoptile (Mocquot et al. 1977 and 1981, Pradet and Bomsel 1978 and Alpi and Beevers 1983), while others studied the relation between ethylene and shoot growth (Musgrave et al. 1972, Vargara et al. 1976, Jack and Campbell 1976, Johnson et al. 1978, Jean et al. 1983, Metraux and Kende 1983 and 1984 and Raskin and Kende 1983 and 1984) or the activity of enzymes under anaerobic metabolism (Opic 1973, Wingnarajah et al.1976 and John and Greenway 1976) and the ultrastructure of mitochondria in particular (Adeeva et al., 1976). In general, it is known that products of fermentation under oxygen deficiency are harmful, if they accumulate in high concentrations in root tisues. However, it was revealed (Bertini et al. 1980, Chirkova 1978 and Alpi and Beevers 1983) that ethanol produced by anaerobic roots of rice is rejected into the root medium so that the level of ethanol in the roots was kept constant. Interestingly, similar results to the above were also found in oat roots (Alpi and Beevers, 1983).

Among the above, the work most relevant to the present study is, probably, that of John et al. (1974) who found that rice roots could take up nutrient at a greater rate if they were pretreated in an anaerobic condition for a period of time. Transferring plants from aerobic to anaerobic conditions reduced the uptake and the reverse was true when anaerobically grown plants were transferred to aerobic conditions. This evidence suggests that uptake of ions into roots is metabolic-linked, and that there may be a special function in the roots operating more

efficiently when they are subjected to oxygen deficiency than under normal conditions. The initial fall or rise of the uptake after changing the root conditions may associate with a transition period between two functions; one of which operates under different conditions. The change of the uptake may be explained in terms of electrical potentials or ionic fluxes but no electrophysiological studies of rice seedlings appear to have been conducted. One of the reason for chosing rice seedlings for this study is to investigate the effect of change in oxygen conditions of the root medium on ion transport mechanism. This, however, requires the development of satisfactory methods of investigation of intact roots.

1.5 An Outline of The Study

The purpose of the present study may be summarised in a series of questions to which answers have been sought.

Consider a portion of an intact root, growing under normal conditions:

(1) Can the flow of a substance(i.e. ionic potassium) between the root portion under investigation and its surroundings be determined by tracer methods or other techniques?

(2) Can the flow to and from the external medium be distinguished from those to and from the rest of the plant (via the xylem or other pathways)?

(3) Can a model of the root system be developed which is simple enough to be handled mathematically, and is realistic enough to give useful information about the amounts of the substance in the various cellular compartments and the fluxes between them? A simple model requires that the root portion under consideration should be as homogenious as possible and should not include a range of zones (i.e. meristem, elongating and mature).

(4) Can the parameters of the root system determined in these investigations be used to indicate the pathways along which the substance is moving passively and those in which active transport is involved?

In this laboratory, previous work has dealt with

flux studies in uniform cortical cells, and provided solutions for steady state and non-steady state conditions of tissues (Pallaghy and Scott, 1969). The present study is, therefore, to extend the previous work by including flux into the xylem utilising the simplified model of ion transport proposed by Pitman (1971). The analytical procedures which assume that the tissue is in a steady state or non-steady state are shown in chapter 2. They are separated into 2 parts; one for the case when ions in the xylem exudate are returned to the bathing medium during washing out, and the other when they are transported into the shoot. This makes it possible to measure ionic fluxes of both excised roots and intact roots.

To test the mathematical analysis, primary roots of rice seedlings which were grown in a modified Hoagland solution are used. They will be referred to as "high salt" roots when compared to work from others whose roots are grown in a $CaSO_4$ solution. In order to measure the fluxes from uniform cells, knowledge of root structure is required. This knowledge, information of growth and how ions are distributed along the root are studied in chapter 3.

The information about root growth and structure is used to define a region of mature or relatively uniform tissue in the intact root for detailed study. In chapter 4 investigations of ion uptake and fluxes are reported. In most experiments, ^{86}Rb is used as a tracer for K^+ ions, thus it is necessary to test whether it is a suitable tracer. This is done in double label experiments. Measurements of ion efflux from the xylem are also attempted. Work similar to that in intact roots is done with excised root segments, in chapter 5. This is to compare how ion movements differ after the shoot and seed are removed. The study also compares excised root tips to mature root segments.

Chapter 6 is concerned with transmembrane electrical potentials in both intact and excised roots. Due to the fact that rice can grow well under anoxic conditions, the study includes the effect of changes in the oxygen state of the root medium on the potentials. A profile of the potentials from the epidermal to the outer layers of cortical cells is

investigated. The xylem potential is also measured.

In an attempt to understand the adaptation of rice roots under anoxic conditions, chapter 7 describes K^+ uptake into roots of young seedlings after the oxygen state of the root environment has been changed. An investigation of the effect of abscisic acid (ABA) on K^+ accumulation in the root and transport into the xylem is included.

Chapter 2

Theoretical Aspects

2.1 Introduction

There has been an increasing number of studies involving ionic flux measurements in higher plant roots during the past 20 years. Mathematical analysis for such studies has been developed from a model considering the whole excised root as an ion accumulation organ which consists of the extracellular space, the cytoplasm and the vacuole (Pitman 1963) to a model including the xylem vessels (Pitman 1971).

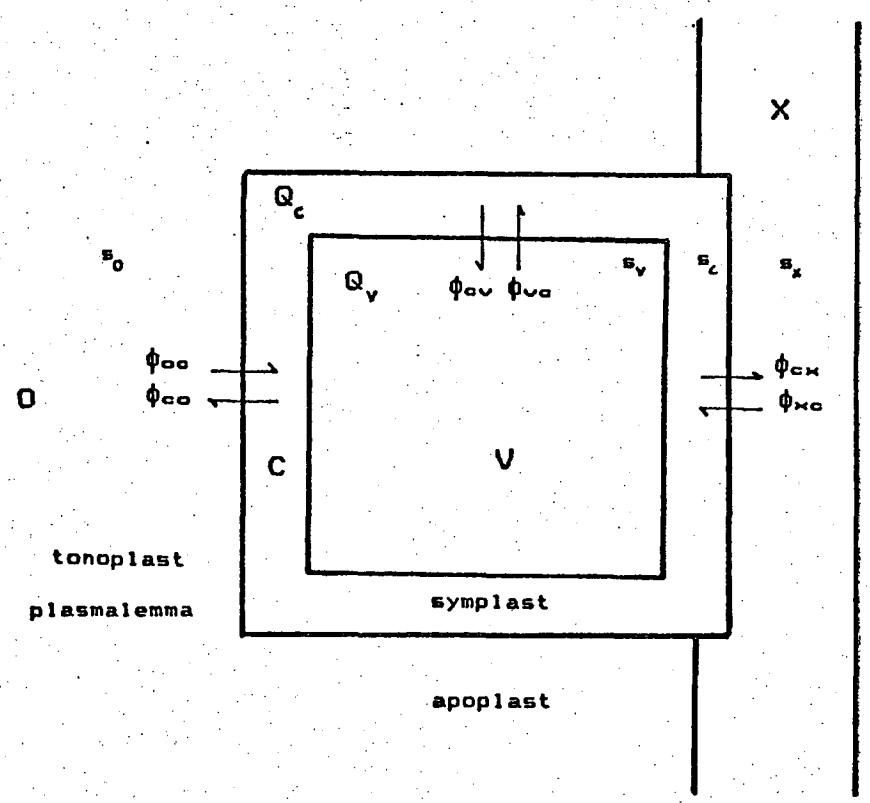
In previous studies, the whole excised root whose tip was attached was treated as if it were a system of uniform cells. However, when K^+ movements in root tip segments were studied (Behl and Jeschke 1982), it was found that fluxes were non-homogeneous. As a consequence, longitudinal symplastic transport between neighbouring cells along the root was included into the Pitman model (1971).

Due to both the finding of some effects of the shoot on root activities (Graham and Bowling 1977, and Bowling 1983), and the question of how the mechanism of ion transport in excised roots differs from that in intact roots (Pitman 1976 and 1982), there was a need to develop a method for flux measurements in intact roots. Besides, investigations on the effect of anoxic conditions on growth of rice have shown that the shoot was an important source of oxygen supply to the root (Barber et al. 1962). One cannot understand how rice roots adjust to such conditions in terms of electrophysiological parameters, unless a suitable method of study has been developed.

To avoid complications from the presence of non-uniform cells at the tip region of the root and to minimise the effect of possible re-translocation of ions via the phloem (Jeschke et al. 1983), the following mathematical analysis will be concerned with the movements of ions across a mature cell region only and the model (see Fig. 2.1) proposed by Pitman (1971) will be utilised.

Fig. 2.1.

The Pitman model of ion transport across plant roots (1971). Showing ionic fluxes across the plasmalemma (ϕ_{oc},ϕ_{co}), the tonoplast (ϕ_{cv},ϕ_{vc}) and the xylem vessels (ϕ_{cx},ϕ_{xc}), and also the content in the cytoplasm (Q_c) and the vacuole (Q_v). s represents the specific activity of tracer ions in the external medium (O), the cytoplasm (C), the vacuole (V) and the xylem (X).



2.2 Tracer analysis of ion movements across the root

Ion uptake by plant roots involves the movement of ions from the external medium (O) to the cytoplasm (C) and the vacuole (V) of root cells. After entering the cytoplasm, they are transported through plasmodesmata channels from the epidermis into the endodermis and, finally, the xylem vessels. The cytoplasmic pathway as depicted is known as the "symplast". Another possible pathway is the "apoplast" where ions from the external medium are transported into the xylem via the intercellular space. After entering the xylem vessels, ions are transported into the shoot by the transpiration stream.

It is widely accepted that under a low external ion concentration (not more than 1 mM), the apoplastic transport of ions is so small, compared to the symplastic transport, that it can be neglected (Anderson 1976, Bowling 1976, Lauchli 1976, and Lüttge and Higinbotham 1979). The transport of ions into the shoot is, therefore, determined mainly by the movements of ions in the symplast. In a region of mature root cells, longitudinal transport of ions along the root via plasmodesmata channels is also possible. This together with the apoplastic transport into the xylem are assumed to be negligible, to simplify the following mathematical analysis. Justification for the assumption will be discussed in chapter 4.

To study ion fluxes across cell membranes, a radioactive tracer of the studied ions is added to the root medium for a period of time. This is followed by washing the tissue in a non-labelled solution. By considering the rate of change of tracer ions in the tissue during loading and washing, ion fluxes across each cell membrane can be estimated.

Assuming a complete mixing of tracer in each compartment during the loading period, the rates of tracer change in the cytoplasm (dY_c/dt) and the whole root (dY/dt) with time are

$$dY_c/dt = (s_o\phi_{oc} + s_v\phi_{vc} + s_x\phi_{xc}) - s_c(\phi_{co} + \phi_{cv} + \phi_{cx}) \qquad (2\text{-}1)$$

$$dY/dt = (s_o\phi_{oc} + s_x\phi_{xc}) - s_c(\phi_{co} + \phi_{cx}) \qquad (2\text{-}2)$$

where s is the specific activity of the tracer in the compartment, and the net uptake into the root is described as

$$(\phi_{oc} - \phi_{co}) = (\phi_{cv} - \phi_{vc}) + (\phi_{cx} - \phi_{xc}) \qquad (2\text{-}3)$$

Since $Y_c = s_c Q_c$, equation (2-2) can be rewritten as

$$Y_c = \left[\frac{(s_o\phi_{oc} + s_x\phi_{xc}) - dY/dt}{(\phi_{co} + \phi_{cx})} \right] \cdot Q_c \qquad (2\text{-}4)$$

The solutions for these equations can be obtained according to the conditions of the root, which are categorized into steady state and non-steady state conditions as follows:

2.2.1 Steady state conditions

For non-growing root cells where Q_c and Q_v are constant, $\phi_{cv} = \phi_{vc}$ and hence $(\phi_{oc} - \phi_{co}) = (\phi_{cx} - \phi_{xc})$. These fluxes are not changing with time. Since the xylem occupies only a small volume of the tissue (i.e. not more than 1.5% of the total tissue volume - see chapter 3 and work by Clarkson et al. 1984), ion content in the xylem (Q_x) should be much less than that in the cytoplasm and the vacuole ($Q_c + Q_v$). If this is the case, $s_x\phi_{xc}$ is always small in comparison with $s_o\phi_{oc}$ so that its rate of change can be neglected. For the large amount of the labelled solution compared to the tissue volume during the loading period, s_o remains effectively constant and equation (2-4) becomes

$$\frac{dY_c}{dt} = - \frac{d^2Y}{dt^2} \cdot \frac{Q_c}{(\phi_{co} + \phi_{cx})}$$

Substituting this equation back to (2-1), then

$$\frac{d^2Y}{dt^2} = \left[(\phi_{co} + \phi_{cv} + \phi_{cx}) \frac{Y_c}{Q_c} - (s_o\phi_{oc} + s_v\phi_{vc} + s_x\phi_{xc}) \right] \cdot \frac{(\phi_{co} + \phi_{cx})}{Q_c}$$

If the ion content in the xylem is negligible in comparison to that in the cytoplasm and the vacuole,

$$Q = Q_c + Q_v .$$

Eliminating s_c, s_v and Y_c, the above equation becomes

$$-\frac{dY^2}{dt^2} + \left[\frac{\phi_{co}+\phi_{cv}+\phi_{cx}}{Q_c} + \frac{\phi_{vc}}{Q_v} \right] \cdot \frac{dY}{dt} + \left[\frac{\phi_{vc}(\phi_{co}+\phi_{cx})}{Q_cQ_v} \right] \cdot Y = (s_o\phi_{oc} + s_x\phi_{xc}) \left[\frac{\phi_{cv}}{Q_c} + \frac{\phi_{vc}}{Q_v} \right] \quad (2\text{-}5)$$

This differential equation can be solved as

$$Y = Y_o + Y_s \exp(-k_s t) + Y_L \exp(-k_L t) \quad (2\text{-}6)$$

where

$$k_s = \frac{\phi_{co} + \phi_{cv} + \phi_{cx}}{2Q_c} + \frac{\phi_{vc}}{2Q_v} + \left[\left(\frac{\phi_{co} + \phi_{cv} + \phi_{cx}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right)^2 - \frac{\phi_{vc}(\phi_{co} + \phi_{cx})}{Q_c Q_v}\right]^{1/2}$$

$$k_L = \frac{\phi_{co} + \phi_{cv} + \phi_{cx}}{2Q_c} + \frac{\phi_{vc}}{2Q_v} - \left[\left(\frac{\phi_{co} + \phi_{cv} + \phi_{cx}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right)^2 - \frac{\phi_{vc}(\phi_{co} + \phi_{cx})}{Q_c Q_v}\right]^{1/2}$$

and

$$Y_o = (s_o\phi_{oc} + s_x\phi_{xc}) \cdot \left[\frac{\phi_{cv}Q_v + \phi_{vc}Q_c}{\phi_{vc}(\phi_{co} + \phi_{cx})}\right]$$

For constant Q_v (i.e. $\phi_{cv} = \phi_{vc}$);

$$Y_o = \frac{(s_o\phi_{oc} + s_x\phi_{xc})\ Q}{\phi_{co} + \phi_{cx}} = \frac{s_o'\phi_{oc}\ Q}{\phi_{co} + \phi_{cx}}$$

where $$s_o' = \frac{s_o + (s_x\phi_{xc})}{\phi_{oc}}$$

Y_s and Y_L can be solved from (2-6) by using boundary conditions during loading. That is when $t = 0$, $Y = 0$

$$\frac{dY}{dt} = s_o'\phi_{oc}$$

when $t =$, $Y = Y_o$

$$Y_s = \frac{s_o'\ \phi_{oc}}{k_s - k_L}\left[\frac{k_L Q}{\phi_{oc} + \phi_{xc}} - 1\right]$$

and

$$Y_L = \frac{s_o'\ \phi_{oc}}{k_s - k_L}\left[1 - \frac{k_s Q}{\phi_{oc} + \phi_{xc}}\right]$$

The general equation for Y during loading becomes

$$Y = \frac{s_o' \phi_{oc}}{k_s - k_L}\left[\left\{1 - \frac{k_L Q}{\phi_{oc}+\phi_{xc}}\right\}\left\{1 - \exp(-k_s t)\right\} + \left\{\frac{k_s Q}{\phi_{oc}+\phi_{xc}} - 1\right\}\left\{1 - \exp(-k_L t)\right\}\right] \quad (2\text{-}7)$$

After loading for a period of time T, the root is transferred to a non-labelled solution for washout. At this moment washout commences and s_o is zero. Hence, the general equation during washout becomes

$$Y = Y_s \exp(-k_s t) + Y_L \exp(-k_L t)$$

where

$$Y_s = \frac{s_o' \phi_{oc}}{k_s - k_L}\left[1 - \frac{k_L Q}{\phi_{oc} + \phi_{xc}}\right]\left[1 - \exp(-k_s T)\right]$$

and

$$Y_L = \frac{s_o' \phi_{oc}}{k_s - k_L}\left[\frac{k_s Q}{\phi_{oc} + \phi_{xc}} - 1\right]\left[1 - \exp(-k_L T)\right]$$

In excised roots, tracer in the xylem is also eluted into the medium during washing. The method for obtaining the short-term (Y_s), long-term (Y_L) components and also the rates of short-term (k_s) and long-term (k_L) exchange from a semi-logarithmic plot of tracer remaining in the tissue against time was described in detail by Walker and Pitman (1976).

The unknown quantities of fluxes and the internal contents can be obtained from the observable quantities of ϕ_{cx}, ϕ_{xc}, and s_o .

Let $$S = Y_s \Big/ \left[1 - \exp(-k_s T)\right]$$

and $$L = Y_L \Big/ \left[1 - \exp(-k_L T)\right]$$

Those quantities are

$$\phi_{oc} = \frac{(k_s S + k_L L)}{s_o'}$$

$$\phi_{co} = \phi_{oc} - (\phi_{cx} - \phi_{xc})$$

$$\phi_{cv} = \phi_{vc} = \frac{k_s k_L}{s_o'} \left[SL(k_s S + k_L L) \right] \left[\frac{(k_s - k_L)^2}{(k_s^2 S + k_L^2 L)^2} \right] \left(1 + \frac{\phi_{xc}}{\phi_{oc}} \right)$$

$$Q_c = \frac{1}{s_o'} \cdot \frac{(k_s S + k_L L)^2}{(k_s^2 + k_L^2 L)} \left(1 + \frac{\phi_{xc}}{\phi_{oc}} \right)$$

$$Q = \frac{(S + L)}{s_o'} \left(1 + \frac{\phi_{xc}}{\phi_{oc}} \right)$$

The above equations can be solved providing that the values of ϕ_{cx} and ϕ_{xc} are obtained from separate experiments. It should be emphasised that these exact solutions are only applicable in cases where there is no net flux across cell membranes.

It is interesting to point out that for isolated cortices, ϕ_{cx} and ϕ_{xc} in the above equations will be diminished and these equations are reduced to the same as those obtained by Pallaghy and Scott (1969). Hence their equations are also applicable for cases when whole segments of roots are used if ϕ_{oc} and ϕ_{co} are corrected for the xylem fluxes.

2.2.2 Non-steady state conditions

Cells are frequently found not to be in the steady state condition as analysed in the previous section. For exaample, Q_v is increasing with time in growing cells. One group of cells may lose ions if the demands of another group are greater. Ionic content may change in response to changes in the environment, and there may be diurnal or circadian changes. Analytical procedure in this section is, therefore, concerned with cases when cells are not in steady state.

If Q_v is a function of time, there are no exact solutions for the differential equation (2-5). An approximate solution can be obtained by treating the short-term and the long-term components separately, as done by Pallaghy and Scott (1969).

During loading, there is initially a rapid build-up of isotope due to a transient change in the cytoplasm, followed by a slower build-up into the vacuole during which s_c has a quasi-steady value. At this stage, Y_c is changing slowly and it is plausible to assume that there is no net flux into the cytoplasm (i.e. $dY_c/dt \simeq 0$). Hence, s_c becomes

$$s_c \simeq \frac{s_o\phi_{oc} + s_v\phi_{vc} + s_x\phi_{xc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}8)$$

and the general equation for tissue loading from (2-2) becomes

$$\frac{dY}{dt} = (s_o\phi_{oc} + s_x\phi_{xc}) - \frac{(\phi_{co} + \phi_{cx})(s_o\phi_{oc} + s_v\phi_{vc} + s_x\phi_{xc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})}$$

$$= (s_o\phi_{oc} + s_x\phi_{xc}) - \frac{(\phi_{co} + \phi_{cx})(s_o\phi_{oc} + s_x\phi_{xc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} - \frac{s_v\phi_{vc}(\phi_{co} + \phi_{cx})}{(\phi_{co} + \phi_{cv} + \phi_{cx})}$$

$$= \frac{\phi_{cv}(s_o\phi_{oc} + s_x\phi_{xc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} - \frac{s_v\phi_{vc}(\phi_{co} + \phi_{cx})}{(\phi_{co} + \phi_{cv} + \phi_{cx})}$$

$$\frac{dY}{dt} = s_o\phi_{in} - \frac{s_v\phi_{vc}(\phi_{co} + \phi_{cx})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} \qquad (2\text{-}9)$$

where

$$\phi_{in} = \frac{\phi_{cv}}{s_o} \cdot \frac{s_o\phi_{oc} + s_x\phi_{xc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}10)$$

During the early state of loading, $s_o >> s_v$ and hence the rate of tracer build-up in the tissue is determined mainly by $s_o\phi_{in}$. For washing out, s_o is made zero. Thus, the second term of equation (2-9) determines the rate of tracer loss from the tissue. The minus sign indicates that tracer content is falling. The equation (2-1) which determines the rate of tracer loss from the cytoplasm becomes

$$\frac{dY_c}{dt} = (s_v\phi_{vc} + s_x\phi_{xc}) - s_c(\phi_{co} + \phi_{cv} + \phi_{cx})$$

$$= - \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{Q_c}\left[Y_c - \frac{(s_v\phi_{vc} + s_x\phi_{xc})\,Q_c}{\phi_{co} + \phi_{cv} + \phi_{cx}}\right]$$

Hence,

$$\frac{dY_c}{\left[Y_c - \frac{(s_v\phi_{vc} + s_x\phi_{xc})\,Q_c}{\phi_{co} + \phi_{cv} + \phi_{cx}}\right]} = - k_s\,dt \qquad (2\text{-}11)$$

where $$k_e = \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{Q_c} \qquad (2\text{-}12)$$

Equation (2-11) can be solved provided that Q_c, s_v and s_x are not functions of time. This assumption is reasonable during the transient period (i.e. when Y_c is changing rapidly). Hence,

$$Y_c - \frac{(s_v\phi_{vc} + s_x\phi_{xc})\ Q_c}{(\phi_{co} + \phi_{cv} + \phi_{cx})} = \exp(-k_e t) \times \text{constant} \qquad (2\text{-}13)$$

At the start of the elution,

$$Y_c = \frac{(s_o\phi_{oc} + s_v\phi_{vc} + s_x\phi_{xc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} \cdot Q_c$$

Hence,

$$\text{constant} = \frac{s_o\phi_{oc}\ Q_c}{\phi_{co} + \phi_{cv} + \phi_{cx}}$$

Rewrite the equation (2-13) as :

$$s_c = \frac{Y_c}{Q_c} = \left[\frac{(s_v\phi_{vc} + s_x\phi_{xc} + s_o\phi_{oc} \exp(-k_e t)}{(\phi_{co} + \phi_{cv} + \phi_{cx})} \right] \qquad (2\text{-}14)$$

Replace s_c in the equation (2-2) and rearrange it as

$$\frac{dY}{dt} = - \frac{(\phi_{co} + \phi_{cx})\left[s_v\phi_{vc} + \ldots + s_o\phi_{oc} \exp(-k_e t)\right] - S_x \Phi_{cv} \Phi_{xc}}{(\phi_{co} + \phi_{cv} + \phi_{cx})}$$

(2-15)

Integration of the exponential term over a period of time large in comparison with its half-time value gives the short-term component (Y_e). That is

$$Y_e = \frac{s_o\phi_{oc}(\phi_{co} + \phi_{cx})}{\phi_{co} + \phi_{cv} + \phi_{cx}} \int_0^{\infty} \exp(-k_e\, t).dt$$

$$= \frac{s_o}{k_e} \cdot \frac{\phi_{oc}(\phi_{co} + \phi_{cx})}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}16)$$

After the initial transient, the rate of tracer loss from the tissue as described in the equation (2-15) can be written as

$$\frac{dY}{dt} = - \frac{(\phi_{co} + \phi_{cx})(s_v\phi_{vc} + s_x\phi_{xc})}{\phi_{co} + \phi_{cv} + \phi_{cx}} + s_x\phi_{xc}$$

$$= \frac{1}{\phi_{co} + \phi_{cv} + \phi_{cx}} \left[s_x\phi_{xc}\phi_{cv} - s_v\phi_{vc}(\phi_{co} + \phi_{cx}) \right]$$

and the rate of long-term exchange (k_L) which is determined from the slope of the semi-logarithmic graph of Y against elution time is

$$k_L = - \frac{d(\ln Y)}{dt} = - \frac{1}{Y} \cdot \frac{dY}{dt}$$

Since $Y = s_cQ_c + s_vQ_v$

replace s_c from the equation (2-8) when s_o is zero, then

$$k_L = \frac{\phi_{vc}(\phi_{co} + \phi_{cx}) - (s_x/s_v)\phi_{xc}\phi_{cv}}{(\phi_{co} + \phi_{cv} + \phi_{cx})Q + \left[(\phi_{vc} - \phi_{co} - \phi_{cv} - \phi_{cx}) + (s_x/s_v)\phi_{xc}\right] Q_c}$$

(2-17)

Since Q_v changes with time, k_L will not be constant during elution. If this is a net loss, k_L will increase

and a semi-logarithmic plot of Y against time (t) will become steeper at large t. The reverse will apply if there is a net gain. Unless this change is very rapid, it is possible to obtain a steady k_L value once the transient has disappeared in the elution graph.

If ϕ_{cx} and ϕ_{xc} are obtained from separate experiments, other fluxes and the internal contents can be estimated. It is reasonable to assume that $s_x\phi_{xc}$ is negligible, since s_x is observed to be much smaller than s_c (see section 4.4.8 in chapter 4). Hence

$$k_L = \frac{\phi_{vc}(\phi_{oc} - \phi_v + \phi_{xc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})Q - (\phi_{oc} + \phi_{xc})Q_c} \qquad (2\text{-}18)$$

To estimate the total tissue content (Q), the above equation is rewritten as

$$Q = \frac{1}{\phi_{oc} + \phi_{vc} + \phi_{xc}} \left[\frac{(\phi_{oc} - \phi_v + \phi_{xc})\phi_{vc}}{k_L} + (\phi_{oc} + \phi_{xc})Q_c \right]$$

The cytoplasmic content (Q_c) can be estimated from equation (2-12) as

$$Q_c = \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{k_s} \qquad (2\text{-}19)$$

Substitute Q_c into the above equation, then

$$Q = \frac{(\phi_{co} + \phi_{cx})\phi_{vc}}{(\phi_{co} + \phi_{cv} + \phi_{cx})k_L} + \frac{\phi_{oc} + \phi_{xc}}{k_s}$$

For small $s_x\phi_{xc}$ compared to $s_o\phi_{oc}$, equation (2-10) becomes

$$\phi_{in} = \frac{\phi_{oc}\phi_{cv}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}20)$$

Substitute ϕ_{cv} into the above equation

$$Q = \frac{\phi_{vc}}{k_L}\left(1 - \frac{\phi_{in}}{\phi_{oc}}\right) + \frac{\phi_{oc} + \phi_{xc}}{k_s} \qquad (2\text{-}21)$$

Note that for $k_L << k_s$, the second term of the equation is negligible relative to the first one. The value of k_L is obtained from a graph of tracer remaining in the tissue against time which is plotted on a semi-logarithmic scale. The value of k_s is determined from a semi-logarithmic plot of tracer loss into the medium during the transient against time.

Analysis for excised roots

During washing out, the amount of tracer found in the solution is from both the xylem vessels and the root surface. It is, therefore, Y_s as shown in equation (2-16). In order to solve the equations for fluxes across each cell membrane, equation (2-10) is rewritten as

$$\phi_{in} = \frac{\phi_{oc}(\phi_v + \phi_{vc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} \qquad (2\text{-}22)$$

Hence,

$$\phi_{cv} = \frac{\phi_{in}(\phi_{co} + \phi_{cv} + \phi_{cx})}{\phi_{oc}} \qquad (2\text{-}23)$$

Let $\alpha = s_o\phi_{in} / k_sY_s$, the equation (2-16) becomes

$$(\phi_{co} + \phi_{cx}) = \frac{\phi_{in}}{\alpha}\,\frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{\phi_{oc}} \qquad (2\text{-}24)$$

By adding (2-23) and (2-24), hence

$$\phi_{oc} = \phi_{in} (1 + 1/\alpha) \qquad (2\text{-}25)$$

Utilising equation (2-3) and ϕ_{in} from the above equation, the equation (2-22) can be rewritten as

$$\phi_{vc} = (\phi_{oc} - \phi_v + \phi_{xc})\alpha - \phi_v$$

$$= (1 + \alpha)(\phi_{in} - \phi_v) + \alpha \phi_{xc} \qquad (2\text{-}26)$$

It should be emphasised that the analysis for both excised and intact roots is essentially concerned only with the amount of tracer in the cytoplasm and the vacuole, and how this changes with time. The amount of tracer in the xylem at any time is assumed to be relatively negligible.

Analysis for intact roots

Unlike excised roots, it is not possible to construct the graph of tracer remaining in the tissue with time from the information of the amount of tracer found in the washout solution alone, since part of the tracer in the tissue is transported into the shoot during the washing. A knowledge of how ion transport into the shoot relates to the total loss from the tissue during the washing out is required.

Equation (2-16) shows that the short-term component (Y_s) can be separated into 2 terms. They represent ion loss into the medium (Y_s') and the xylem (Y_s'') as follows

$$Y_s' = \frac{s_o}{k_s} \cdot \frac{(\phi_{co}\phi_{oc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})} \qquad (2\text{-}27)$$

and

$$Y_s'' = \frac{s_o}{k_s} \cdot \frac{(\phi_{cx}\phi_{oc})}{(\phi_{co} + \phi_{cv} + \phi_{cx})}$$

The total amount of tracer loss into the medium during the transient is related to the total loss from the tissue (Y_s in equation 2-16) as

$$Y_s = \frac{(\phi_{co} + \phi_{cx})}{\phi_{co}} \cdot Y_s' \qquad (2\text{-}28)$$

This is also true for the total amount of tracer which leaves the tissue at the end of washing. That is

$$Y = \frac{(\phi_{co} + \phi_{cx})}{\phi_{co}} \cdot Y' \qquad (2\text{-}29)$$

Rewrite equation (2-27) as

$$\frac{k_s Y_s'}{s_o} = \frac{\phi_{co}\phi_{oc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}30)$$

Substitute ϕ_{oc} from equation (2-20), hence

$$\frac{s_o \phi_{in}}{k_s Y_s'} = \frac{\phi_{cv}}{\phi_{co}}$$

Let $\alpha' = s_o \phi_{in} / k_s Y_s'$

then $\phi_{cv} = \alpha' \phi_{co} \qquad (2\text{-}31)$

Utilise $\phi_{oc} = \phi_{co} + \phi_v + \phi_x$, the equation (2-30) becomes

$$\alpha' \phi_{co}^2 + \left[(\phi_v + \phi_x)\alpha' - (1 + \alpha')\phi_{in} \right] \phi_{co} - \phi_{cx}\phi_{in} = 0 \qquad (2\text{-}32)$$

Let $\gamma = (\phi_v + \phi_x)\alpha' - (1 + \alpha')\phi_{in}$, the solution for the above equation is, then

$$\phi_{co} = \left[\left(\frac{\gamma}{2\alpha'} \right)^2 + \frac{\phi_{cx}\phi_{in}}{\alpha'} \right]^{1/2} - \frac{\gamma}{2\alpha'} \qquad (2\text{-}33)$$

The values for ϕ_{cx}, ϕ_{in} and ϕ_v are obtained from separate experiments.

Equation (2-29) shows that the graph of tracer remaining in the tissue can be constructed after $(\phi_{co} + \phi_{cx}) / \phi_{co}$, which will be referred to as a β factor, is known. Once this graph is obtained, the analysis can be processed as in the previous section.

The specific activity of the cell compartments

At a quasi-steady state of loading, the specific activity in the vacuole and the xylem vessels are small in comparison to that of the cytoplasm. Equation (2-8) becomes

$$s_c \simeq \frac{s_o \phi_{oc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}34)$$

During washing out when s_o is made zero, the specific activity in the cytoplasm at quasi-steady state is determined by

$$s_c \simeq \frac{s_v \phi_{vc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}35)$$

where $s_v = Y_L/Q_v$

The specific activity of the xylem (s_x) cannot be obtained from washout studies. However, it can be calculated from

$$s_x = \frac{dY_x/dt}{dQ_x/dt} \qquad (2\text{-}36)$$

where dY_x/dt is the rate of tracer build-up in the upper part of the plant, beyond the labelled portion of the root and dQ_x/dt is that of ion transport into the xylem. The latter can be obtained by analysing ion content in the upper part of the plant at various time interval.

2.3 Discussion

The above solutions for excised aand intact roots for steady state and non-steady state conditions are summarised in Table 2.1. It is interesting to note that the analysis of fluxes in root tissue in which xylem fluxes are taken into account is very similar to that for a simple three compartmental model for isolated cortices (Pallaghy and Scott 1969). The minor differences take into account the fact that the root tissue is exchanging tracer both with the external solution and the xylem. However, when ϕ_{xc} is relatively negligible (Pitman 1971, Jeschke 1973, Davis and Higinbotham 1976, Behl and Jeschke 1982 and Jeschke 1982), the influx into the tissue is from the external medium alone while the efflux from the tissue is via the root surface (ϕ_{co}) and the xylem (ϕ_{cx}).

It should be emphasised that the Pitman model can be utilised for the case when a mature cell region of intact roots is used providing that longitudinal transport between neighbouring cells in the cortex can be neglected. This together with the assumptions used in mathematical analysis are to be considered in chapter 4.

Table 2.1

A summary of equations for ion fluxes across the plasmalemma (ϕ_{oc} and ϕ_{co}) and the tonoplast (ϕ_{cv} and ϕ_{vc}) and the internal ion contents (Q_c and Q) when transport into the xylem is taken into account.

(a) For steady state tissues

(b) For non-steady state tissues.

(a) Steady State Conditions

$$\phi_{oc} = \frac{(k_e S + k_L L)}{s_o'}$$

$$\phi_{co} = \phi_{oc} - (\phi_{cx} - \phi_{xc})$$

$$\phi_{cv} = \phi_{vc} = \frac{k_e k_L}{s_o'}\left[SL(k_e S + k_L L)\right]\left[\frac{(k_e - k_L)^2}{(k_e^2 S + k_L^2 L)^2}\right]\left(1 + \frac{\phi_{xc}}{\phi_{oc}}\right)$$

$$Q_c = \frac{1}{s_o'} \cdot \frac{(k_e S + k_L L)^2}{(k_e^2 + k_L^2 L)}\left(1 + \frac{\phi_{xc}}{\phi_{oc}}\right) \quad \text{where} \quad S = Y_e \,/\, 1 - \exp(-k_e T)$$

$$L = Y_L \,/\, 1 - \exp(-k_L T)$$

$$Q = \frac{(S + L)}{s_o'}\left(1 + \frac{\phi_{xc}}{\phi_{oc}}\right) \quad \text{and} \quad s_o' = s_o + \frac{\phi_{xc}}{\phi_{oc}} \cdot s_x$$

(b) Non-Steady State Conditions

Excised roots

$$\phi_{oc} = \phi_{in}(1 + 1/\alpha)$$

$$\phi_{co} = \phi_{oc} - \phi_v - \phi_x$$

$$\phi_{vc} = (1 + \alpha)(\phi_{in} - \phi_v) + \alpha\phi_{xc}$$

$$Q_c = \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{k_e}$$

$$Q = \frac{\phi_{vc}}{k_L}\left(1 - \frac{\phi_{in}}{\phi_{oc}}\right) + \frac{\phi_{oc} + \phi_{xc}}{k_e}$$

where $\alpha = s_o\phi_{in} / k_e Y_e$

$$\phi_{in} = \frac{\phi_{oc}\phi_{cv}}{\phi_{co} + \phi_{cv} + \phi_{cx}}$$

$$\phi_v = (\phi_{cv} - \phi_{vc})$$

and $\phi_x = (\phi_{cx} - \phi_{xc})$

Intact roots

$$\phi_{co} = \left[\left(\frac{\gamma'}{2\alpha'}\right)^2 + \frac{\phi_{cx}\phi_{in}}{\alpha'}\right]^{1/2} - \frac{\gamma'}{2\alpha'}$$

$$\phi_{cv} = \alpha'\phi_{co}$$

$$\phi_{oc} = \phi_{co} + \phi_v + \phi_x$$

where

$$\alpha' = s_o\phi_{in} / k_e Y_e'$$

and $\gamma' = (\phi_v + \phi_x)\alpha' - (1 + \alpha')\phi_{in}$

Chapter 3

Preliminary Studies of Growth, Root Structure and Ion Uptake

3.1 Introduction

As is known, the mechanism of ion transport in plants as a whole is rather complex due to the involvement of several tissues and organs in the process. Scientists have attempted to understand the fundamentals of the transport by estimating the net inflow of substances which are essential for plant growth and following their pathways. The immediate organ involved in the study is the root, since it is in direct contact with the external source of nutrients.

Generally, mineral requirements for plants vary from species to species and the behaviour depends on the environment in which they are grown. Higher K^+ than Na^+ content seems to be the characteristics of glycophytes (Pitman 1965a, Scott et al. 1968 and Jeschke et al. 1983), while the reverse is true for halophytes (Anderson et al. 1977 and Ginzburg 1981). In some plant species such as barley, Ca^{+2} ions in the root medium inhibits Na^+ (Bange 1979) and K^+ (Rain and Epstein 1967) uptake, but enhances K^+ uptake in rice (Zsoldos and Karvaly 1978).

In the laboratory, culture solutions are chosen and these vary according to the nature of the plant and the purpose of the investigations. Since later chapters will be concerned with studies in high salt roots, it is of interest to observe the growth of rice seedlings which are grown in the same culture solution throughout their life. It is also important to know the state of mineral content in these seedlings, in general, and how minerals are distributed along the root and in the whole plant. This information is essential for the work in later chapters when an appropriate region of the root is chosen for flux studies (chapter 4 and 5).

3.2 Experimental Materials

The culture solution used for growing rice seedlings in this study was adopted from Etherton and Higinbotham (1960). It was composed of, in mM; 1.0 KCl, 0.905 NaH_2PO_4, 0.48

Na_2HPO_4, 1.0 $Ca(NO_3)_2$ and 0.25 $MgSO_4$. Each of these components was prepared in bulk at 0.1 M concentration and the required quantity of each kind was diluted for use.

For convenience, this culture solution will be referred to as a 1x solution throughout this study. Its pH value was between 5.5 - 5.6.

Rice seeds (Oryza sativa cv. Calrose) used throughout this study and in the later chapters were supplied by the Rice Marketing Board, N.S.W.

3.3 Experimental Methods

3.3.1 Rice growing in the laboratory

After seeds were soaked in distilled water for 2 days at 25°C temperature, most of the seed coat was ruptured and a white coleoptile was emerging. At first, they were grown between moist filter papers of which one end was immersed in a 1x solution. It was found that the roots were not straight and were difficult to deal with in the later experiments.

A method of growing in a hydroponic system was used instead. Rice seeds, after 2 days soaking in distilled water, were pushed into U-shaped holes made in a perspex bar (see Fig. 3.1). The awn was inward and the embryo faced downward. The perspex bar was placed over a plastic container which contained about 1 litre of the 1x solution. The level of the solution was up to the seeds. Aeration was supplied throughout plant growth. About 100 plants were grown at a time. The whole system was kept in controlled conditions at 25°C in the dark. On the 3rd day of growing, retarded seedlings which grew unusually slowly compared to others were discarded.

3.3.2 Determination of the internal ion content

The investigation of the distribution of ions along the root was carried out after washing the root in distilled water for 5 min to remove ions in the free space. Seedlings at 4 days and 6 days old were used and 10 seedlings

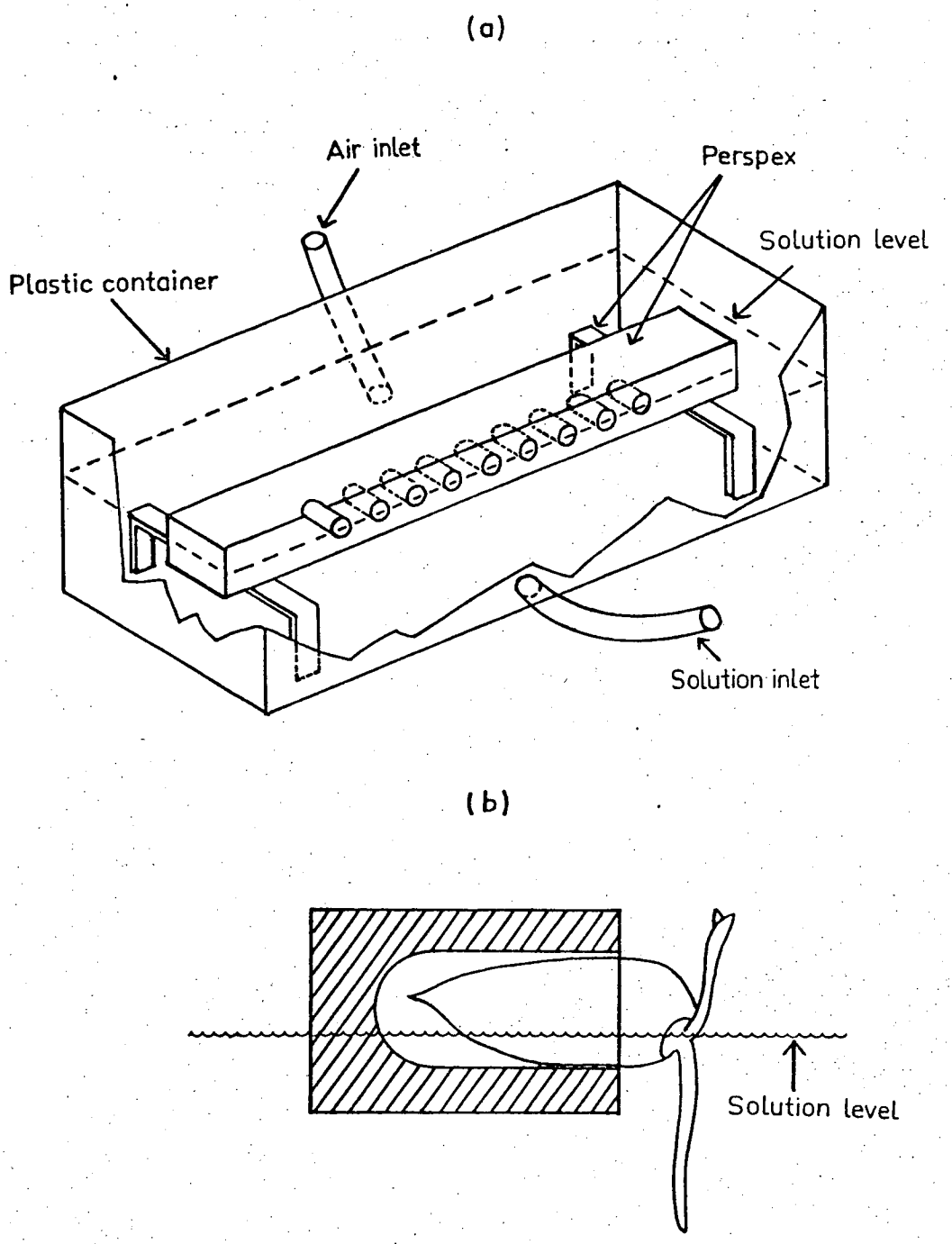


Fig. 3.1

A diagram of rice growing in the laboratory. Rice seeds were pushed in U-shape holes, which were drilled in a perspex bar, in such a way that the embryo was outside and facing down to the culture solution. The bar was placed over a 1 litre culture solution with a continuous aeration throughout the plant growth. The whole system was kept in a controlled environment of 25° C temperature in the dark.

were taken for each observation. After washing, the roots were blotted with filter paper and excised into 2 mm segments upto 20 mm from the tip. The segments were boiled in distilled water for 1 hour. The extracted solution was collected and the tissue was reboiled in distilled water for another 30 min before being discarded.

The method of analysing the internal ion content in the tissue is separated into two parts; one for cations and the other for anions. Of the anions, only Cl^- ions were analysed.

3.3.2.1 The determination of cations

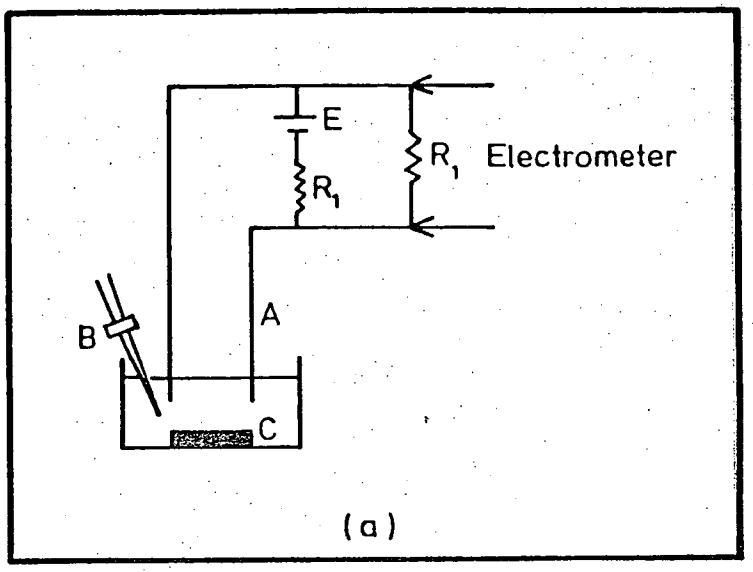
For cation determination, the extracted solution obtained by boiling root segments was diluted to a known volume and analysed by using an Atomic Absorption Spectrophotometer, Varian Techtron AA-175 series model. Ion species which were analysed were K^+, Na^+, Mg^{+2} and Ca^{+2}.

If the total content in whole plants was to be analysed, excision was made such that the seed was separated from the root and the shoot. Each sample of tissue was boiled in a weak HNO_3 solution of 0.1 N concentration for 30 min and the tissues were rinsed in distilled water twice. The extracts were combined and analysed for the content.

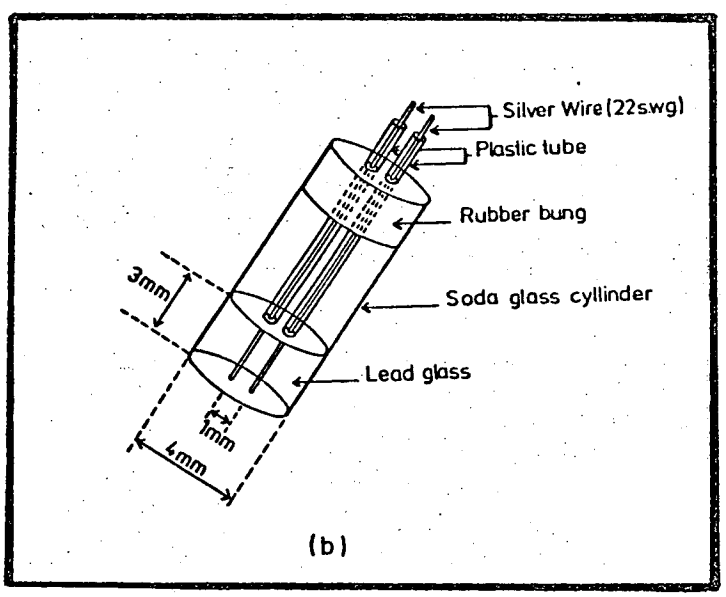
3.3.2.2 The determination for Cl^- ions

After extracting ions from the tissue (section 3.3.2), the extract was evaporated to less than 0.5 ml. After the sample was completely cool, 0.4 ml of 0.5 N HNO_3 solution was added plus sufficient A.R. acetone to make up a 2 ml sample. The final sample contained 0.1 N HNO_3 and 60-70% acetone. It was titrated with a freshly prepared 0.1 mM $AgNO_3$ solution, using a 1 ml burette with 100 divisions. Estimation of Cl^- followed the method of Bishop and Dhaneshwar (1962).

Fig. 3.2 shows the equipment used for Cl^- titration. A small current of 10^{-8} amperes was passed through the wires, creating a potential difference between two electrodes which was observed by using a high input impedance electrometer



(a) Showing an arrangement for chloride titration. E = 10 volts, $R_1 = 10^7$ ohms and $R_2 = 10^9$ ohms. A represents a silver electrode (see Fig. b), B is a burette and C a micromagnetic stirrer.



(b) A diagram of silver electrodes constructed in this laboratory, following Bishop and Dhaneshwar (1962a and b). Each end of both silver wires was embedded in lead glass which sealed completely one end of a soda glass cylinder. The embedding allowed only the surface area of the wire to be exposed to a sample containing Cl^- ions. The other end of the wire was covered with a small plastic tube to keep the two wires insulated. The whole apparatus was about 50 mm long and 4 mm wide.

The cross-section area of the silver wire was 0.0039 $(cm)^2$.

Fig. 3.2

with a chart recorder. As an amount of $AgNO_3$ solution was added to the 2 ml sample, the potential difference between the electrodes increased as shown in Fig. 3.3. The increase was slow at the beginning. However, when the amount of $AgNO_3$ solution added to the sample was about 80% of the end point volume, a steep rise in the potential difference occurred. The magnitude was about 75% of the total potential change. The titration end point was indicated by the sharp fall of the potential difference. As the commencement of the fall was observed, the total amount of $AgNO_3$ solution used was recorded.

To facilitate a thorough mixing between the sample and the titrant solution, a micromagnetic stirrer was used. It was spinning throughout the titration period. Since the speed of the stirrer affected the potential reading, it was fixed at a particular rate throughout the measurements.

In a preliminary experiment with a sample of known concentration, the error was found to be between 1-4%, depending on the speed of adding $AgNO_3$ solution. The solution was added at a quicker rate at the beginning of the titration and at a slower rate after the potential had changed more than 50%. The tip of the burette was immersed in the sample solution throughout the titration, which took only 3-5 minutes. Cl^- concentrations not less than the order of 10^{-6} molar can be simply determined with this equipment. It was noted that the sharpness of the rise and fall of the potential was reduced, making it difficult to distinguish the turning point, if a sample with concentration smaller than the above was used.

After each titration, the electrode was cleaned by using an ultrasonic bath. Immersing the electrode tip into a container of distilled water for one minute was adequate for cleaning.

In some experiments when the chloride content of whole tissues of roots, seeds and shoots were to be determined, the tissues were boiled in a 0.1M NaOH solution. It was found that the electrodes were less sensitive in this case. It was observed that the rise and fall of the potential did not occur as sharply as before and this made it hard to ascertain when the end point had been reached. Due to this problem, boiling tissues in

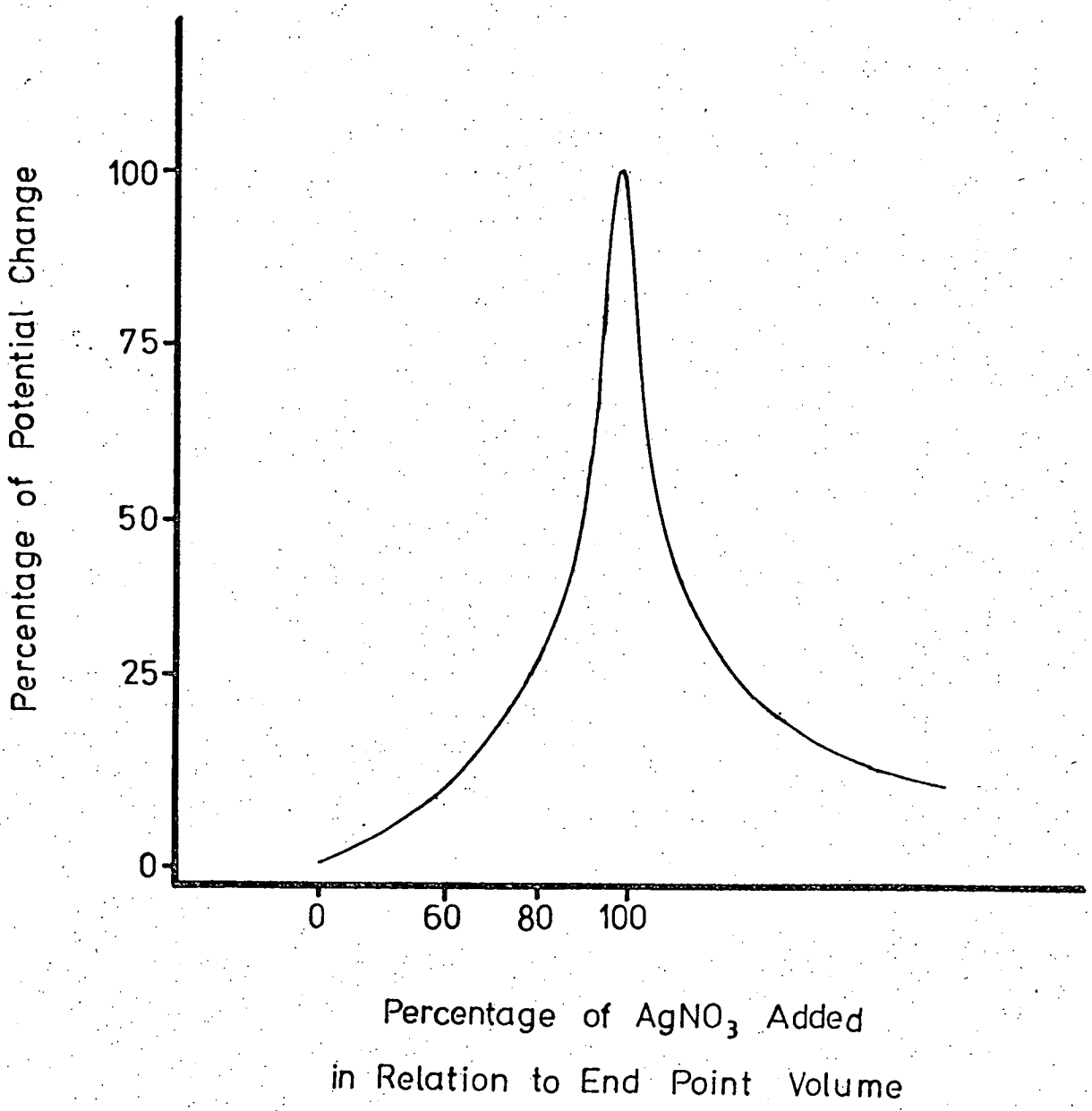


Fig. 3.3

Showing the potential difference between two electrodes as a volume of $AgNO_3$ solution was added to a Cl^--containing solution. The titration end point is indicated by the sharp reversal of the potential. Only the last 20% of $AgNO_3$ volume required for end point potential accounts for 75% change in the potential.

distilled water was preferred.

3.4 Results

3.4.1 Plant growth rates

In order to estimate the rate of ion accumulation in root cells (section 3.4.4), knowledge of the rate of root growth is required. The root and shoot length of seedlings grown in the hydroponic system in the dark, as described in section 3.3.1, were measured from the second day to sixth day of growing. To avoid the effect of light on plant growth, once the seedlings were taken out from the growing system they were discarded except those at 4 days and 6 days old, which the roots were used to analyse for ion content. The measurements were, therefore, made on different plants from the same batch. Although most of the roots germinated after the first day of growing, the length was measurable on the 2th day.

The result is shown in Fig. 3.4, for 40 plants taken from 4 batches. The limit is $\pm$ S.E. It appears that plant growth during the first 2 days is at a smaller rate. After the 3rd day of growing, root and shoot are growing at steady rates. Taken between 3-6 days of growing, the growth rates are 0.6 mm.hr^{-1} for the root and 0.2 mm.hr^{-1} for the shoot. Although the coleoptile germinated before the root, its rate of growth was much smaller. It is possible that during the 2 days of soaking the seed, root growth was inhibited due to being in a non-aerated medium. The inhibition of root growth under anoxic conditions without affecting coleoptile growth was reported by Opic (1973) and Vartapetian et al. (1978). In comparison to other plant species, the rate of root growth of rice is slightly smaller than broad bean (Scott et al. 1968) and is half of the rate for barley roots (Weisenseel et al. 1979).

It was observed that on the 4th day of growing, the first leaf emerged from the coleoptile and on the 5th day

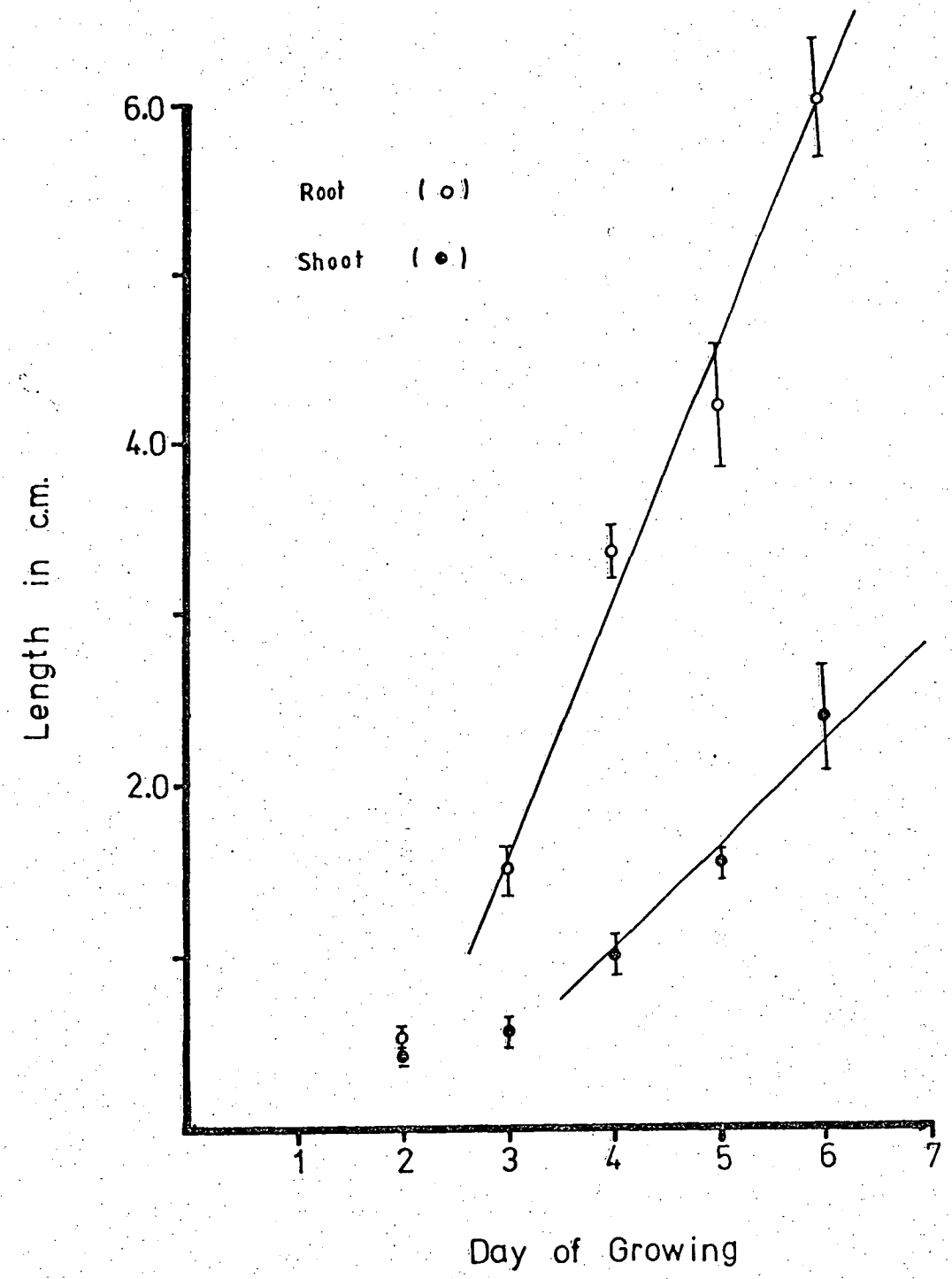


Fig. 3.4

Showing the rate of root (o) and shoot (•) growth in a hydroponic system which was kept in the dark at 25° C. The limit indicates the standard error of the means for 40 plants, taken from 4 batches.

adventitious roots and the lateral roots appeared. Lateral roots started at the basal part of the root and germinated down to about 30 mm from the tip by the 7th day. Root hairs started at about 2.5 mm from the tip.

3.4.2 The growth and structure of roots

This section reports the growth and structure of roots using microtomed sections. Roots at 6 days were excised into 5 mm segments up 30 mm from the tip. Each 5 mm of these segments was treated separately using the method of Johansen (1940) for killing and fixing. After embedding in paraffin wax, they were microtomed into 10 μm thick sections, mounted and examined by light microscopy.

Plate 3-1(a) and 3-2 show cross sections of rice roots at regions between 15-20 mm and 25-30 mm from the root tip. To differentiate the xylem vessels in the stele, acid fushsin stained segments were counterstained with iodine green. As can be seen, most of the root area is occupied by cortical cells. There is a clear differentiation between the cortex and the endodermis. The xylem vessels are shown clearly in these regions of the root. However, it is not possible to know whether they are living. Vessels could also be observed in sections between 5-10 mm from the tip. The section between 25-30 mm from the tip shows an eruption of a lateral root from the stele into the root cortex and some development of large spaces in the cortex along the radial direction, between the outer and the inner cortical cells. Note that this development does not exist in the section between 15-20 mm from the tip. They could possibly be air spaces which are developed when the roots become mature (see discussion).

The geometrical characteristics of the root between 15-20 mm from the tip are shown in table 3.1. Note that the central cavity could also be observed in sections between 5-10 mm from the tip. At distance between 15-20 mm from the tip, the cavity is about 6% of the tissue area.

The same method was employed to obtain longitudinal sections. Table 3.2 shows the size of cortical cells along 30 mm root length. As is seen, cell elongation takes place

Plate 3-1.

(a) A cross section of a portion of a mature rice root, between 15-20 mm from the tip. Xylem vessels, the endodermis, cortical cells and air spaces are shown.

(b) A longitudinal section of a portion of a rice root tip. Vacuolated cells start to form at about the fifth cell back from the extreme tip, a distance of 35 μm from the root apex.

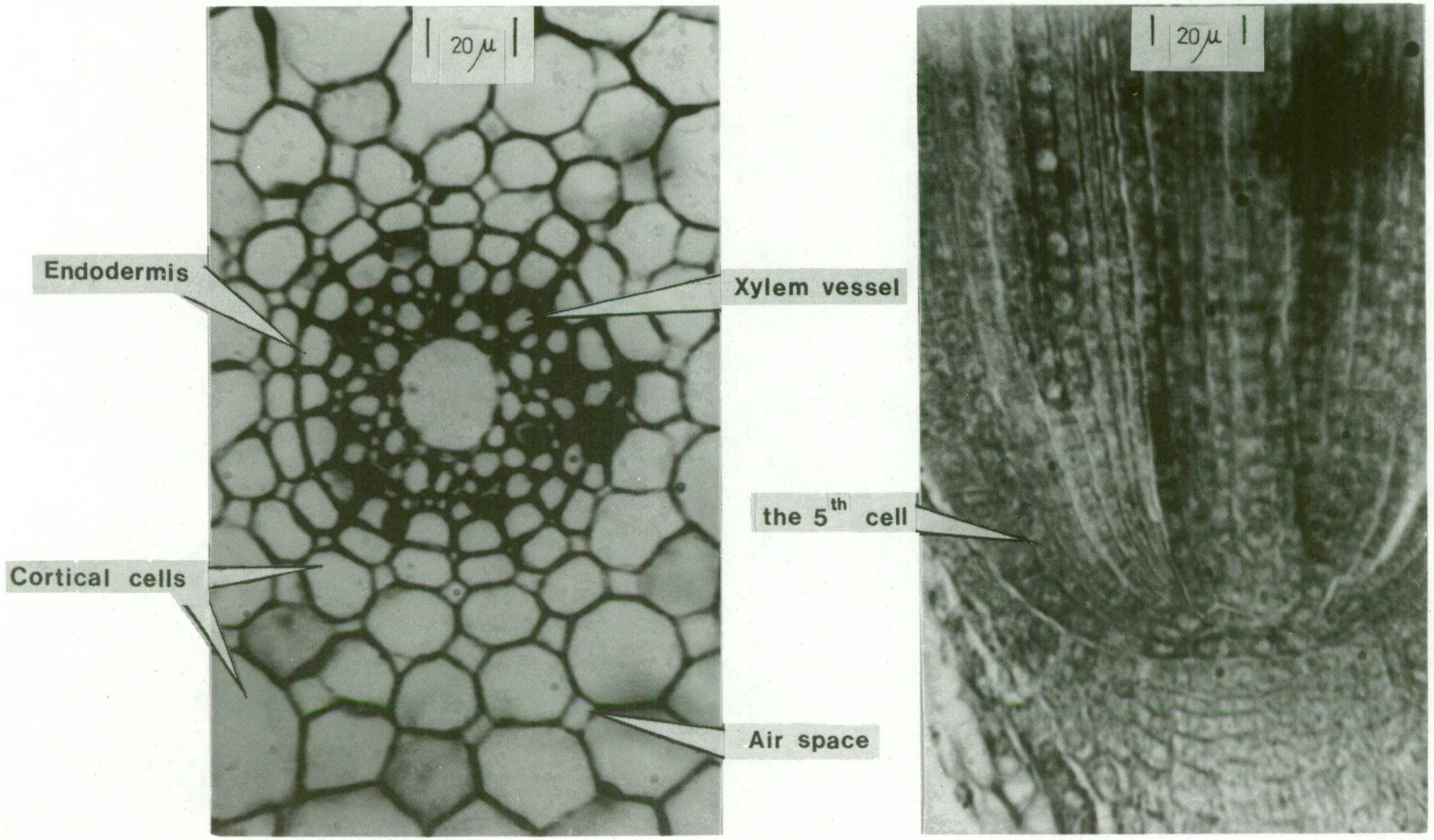
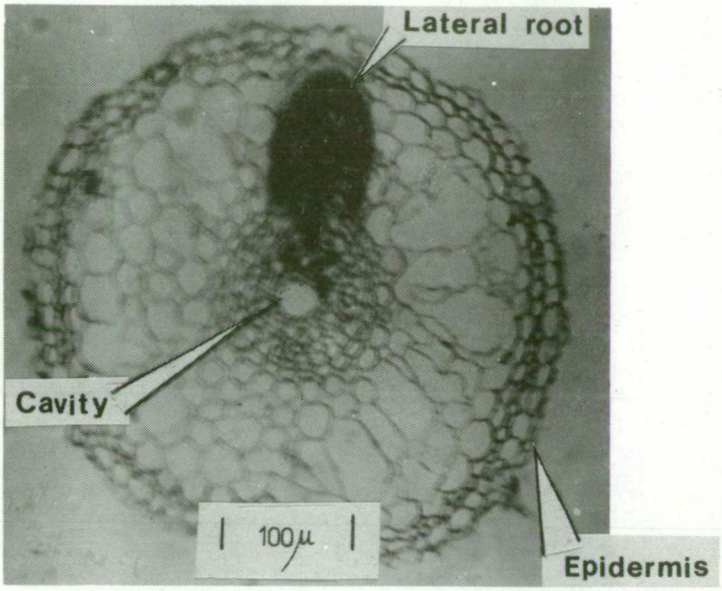
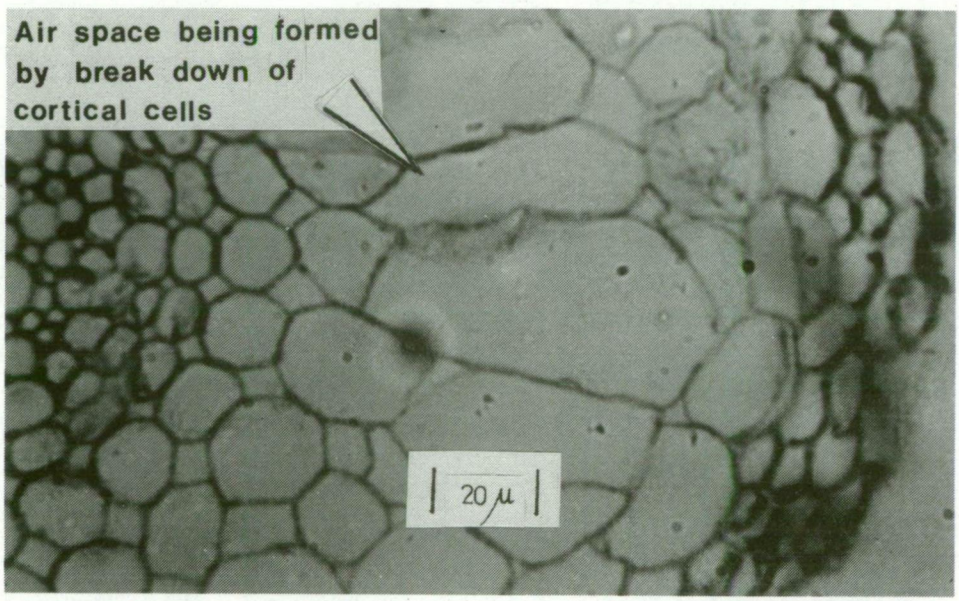

20μ
Endodermis
Xylem vessel
Cortical cells
Air space
20μ
the 5th cell
(a)
(b)

Plate 3-1.



(a)



(b)

Plate 3-2.

A cross section of a rice root between 25-30 mm from the tip. Lateral roots (a) have started to form in this region and large air spaces (b) are formed by break down of cortical cells (see discussion).

Table 3.1

Geometrical characteristics of rice roots at 6 days old, observed through a light microscope at 100x magnification. The observation was made from 12 cross sections of 10 μm thick, at a region between 15-20 mm from the root tip.

	μm
Root diameter	393 ± 13
Diameter of the stele	108.0 ± 5
Width of epidermis	25.1 ± 1
Width of cortex	116.3 ± 4
Diameter of the cavity	24 ± .1

Table 3.2

Showing the size of cortical cells along 30 mm from the tip. The limit is standard deviation of 200 cells, taken from 10 longitudinal sections.

Note that the 5th cell is least vacuolated cell at a distance about 35 μm from the tip (not including the root cap).

Distance from the tip (mm)	Length μm	Width μm
5th cell	9 ± .3	8 ± .1
0 - 1	17 ± 2.8	12 ± .2
1 - 5	56 ± 1.2	18 ± .2
5 - 10	73 ± 1.7	19 ± .2
10 - 15	85 ± 1.7	-
15 - 20	89 ± 1.7	-
20 - 25	94 ± 1.9	-
25 - 30	98 ± 1.9	-

rapidly from 9μm close to the tip to 56μm at the distance about 5 mm away from it. Within this region, radial expansion of the cells also takes place. The meristematic region of the root is between 0-1 mm from the tip. The expansion and elongation of cells between 5 mm and 10 mm from the tip are relatively slow. Note that cell expansion ceases after 5 mm from the tip, while cell elongation still takes place at distances greater than 10 mm from the tip, but at a much smaller rate. The cells arising in the meristem are non-vacuolated upto the 5th cell from the root apex (see Plate 3-1b).

3.4.3 Ion content along the root

Work in this section is to determine the internal content of K^+, Na^+, Mg^{+2}, Ca^{+2} and Cl^- ions along the terminal of 20 mm of roots of 4 day and 6 day-old seedlings which were grown in the dark. The methods for estimating cations and Cl^- contents in root segments was described in section 3.3.2. The results are shown in Fig. 3.5(a) and (b) for 4 day and 6 day old roots, respectively. For both plant ages, K^+ appears to be most concentrated. The amount of Cl^- is greater than of Na^+ and Mg^{+2}. It should be noted that the average Ca^{+2} concentration in a whole root was observed to be about 96 m.equiv.kg^{-1} fresh weight on the 4th day, but no variation along the root was detected.

On the 4th day of growing, the distribution of K^+ is such that the content at the meristematic region (0-5 mm from the tip) is smaller than in the upper part of the root and fairly constant. Above this region, the content increases gradually and between 13-19 mm from the tip it becomes fairly constant. Slightly different from K^+, Cl^- content increases from the tip toward the mature cell region and the constant level of Cl^- content is reached at about 7 mm behind the tip. There is no significant difference in the amount in moles between Na^+ and Mg^{+2} ions. Unlike the other two ion species measured, the distribution along the root of Na^+ and Mg^{+2} appears to be at steady levels.

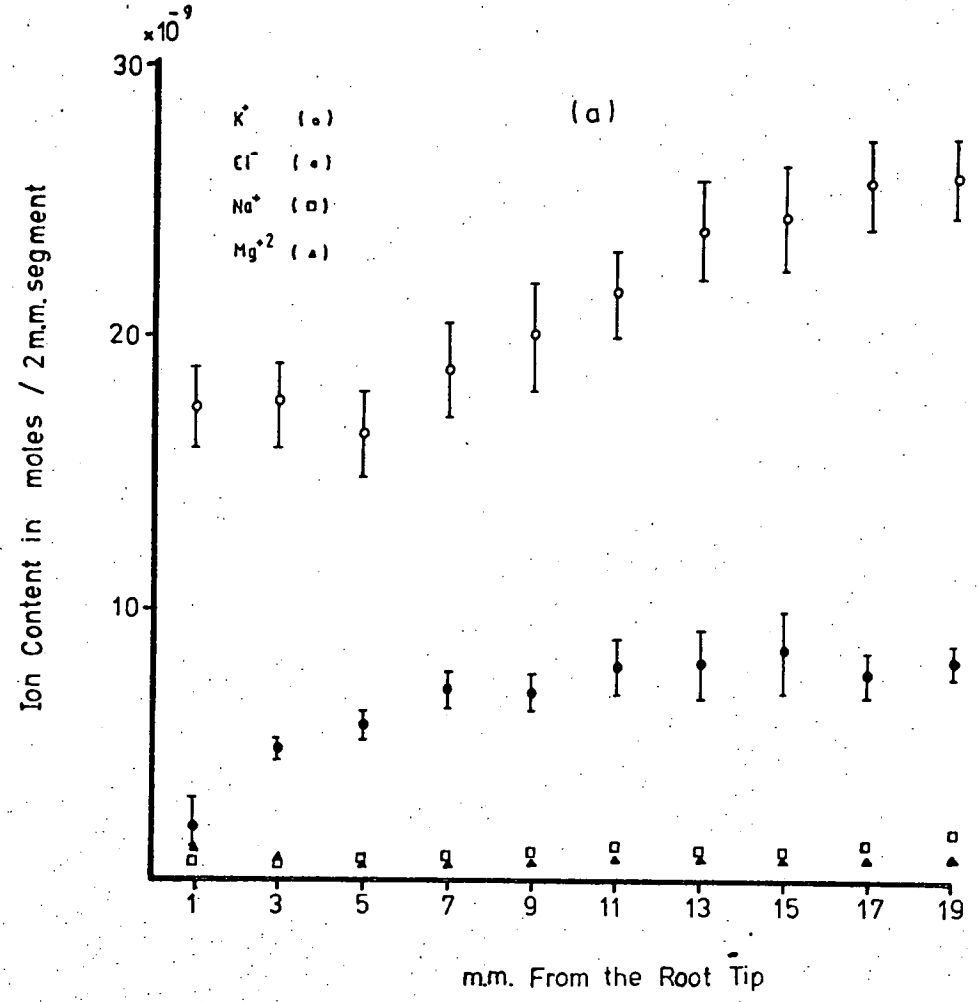
On the 6th day of growing, Fig. 3.5(b) shows that ion content in the new grown root cells remains constant for Cl^-,

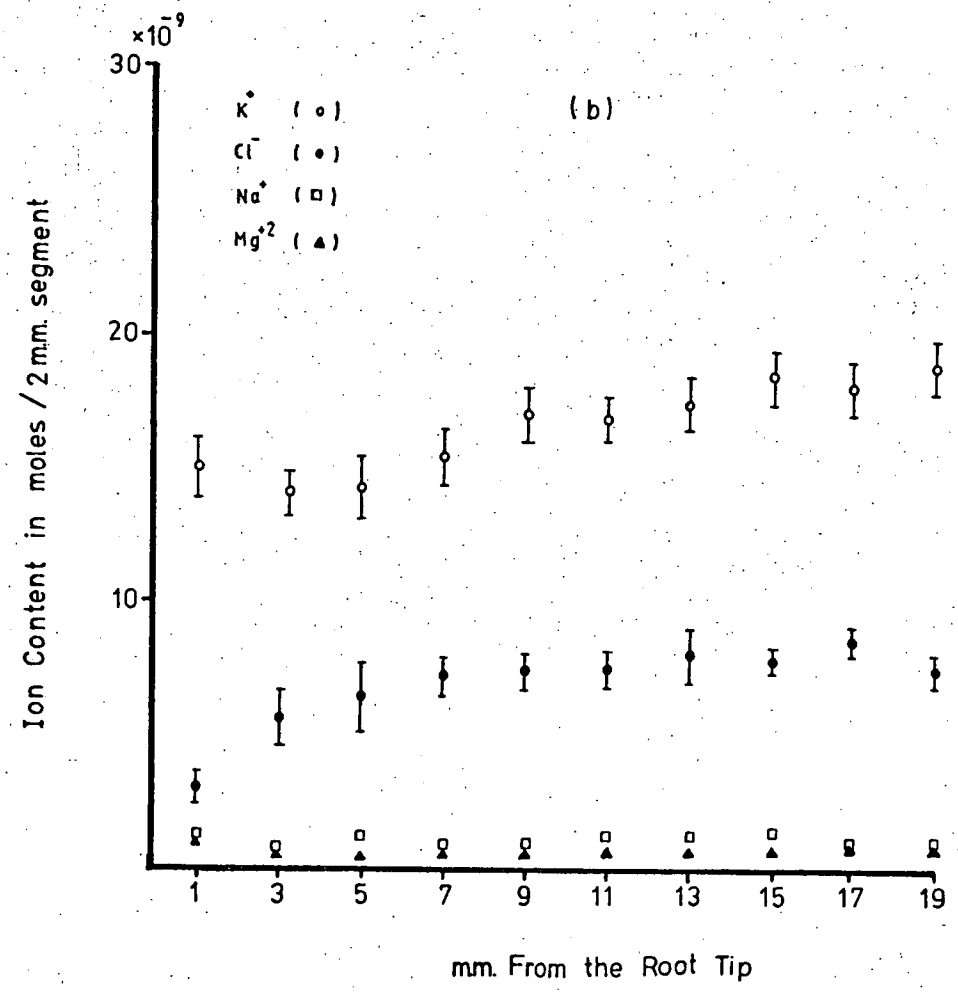
Fig. 3.5

The distribution of K^+, Na^+, Mg^{+2} and Cl^- ions along 20 mm roots grown in the dark at 25° C temperature. The limit denotes the standard error of the mean of 6 observations for cations and 3 observations for Cl^- ions. Ten seedlings were used in each observation.

(a) Showing the distribution along the root of 4 day old seedlings

(b) The distribution at 2 days later.


×10⁻⁹
30
20
10
K⁺ (o)
Cl⁻ (•)
Na⁺ (□)
Mg⁺² (▲)
(a)
Ion Content in moles / 2 m.m. segment
1
3
5
7
9
11
13
15
17
19
m.m. From the Root Tip


×10⁻⁹
30
20
10
K⁺ (o)
Cl⁻ (•)
Na⁺ (□)
Mg⁺² (▲)
(b)
Ion Content in moles / 2 m.m. segment
1
3
5
7
9
11
13
15
17
19
mm. From the Root Tip

Na^+ and Mg^{+2} ions and the distribution of ions along the root is similar to that of 4 day roots. At comparable distances from the root tip, the K^+ content is less at 6 days than at 4 days suggesting the possibility of a shortage of the supply from the seed

When the size of the root was observed under a microscope (section 3.4.4), it was found that the new growing part of the root became thinner with time (see Fig. 3.7). Since K^+ is essential for growth and cell division (Luttge and Higinbotham 1979), the smaller size of the root with age supports the possibility of inadequate supply of K^+ into the new grown root during this period.

Since the root has more K^+ than other cations, it is of interest to investigate the cation distribution in the whole plant. Seedlings at the age of 3 days to 6 days were analysed for K^+ content by separating the seed from the root and the shoot. Each group of tissue was weighed and boiled in a 0.1N HNO_3 solution and the method employed was the same as described in section 3.3.2.1. The time course of K^+ ions in the seed, root, shoot and in the whole plant, expressed in $m.equiv.kg^{-1}$ tissue fresh weight are shown in Fig. 3.6. As is seen, the concentration in the seed decreases with age. Although there is some variability, the average concentration in the root seems to remain constant. That in the shoot decreases slightly with age. In total, the concentration of the whole plant remains fairly constant.

3.4.4 The rate of net accumulation of K^+ and Cl^- into a group of cells in the root

As the root grows, individual cells become further from the root tip. It is possible to estimate the net ion flux into a group of cells, following the method of Scott et al. (1968).

The rate of net ion accumulation (ϕ) is determined in relation to the rate of change of content at a constant distance from the tip ($\partial q/\partial t$) and the rate of change of content with position along a root of a particular age ($\partial q/\partial x$)

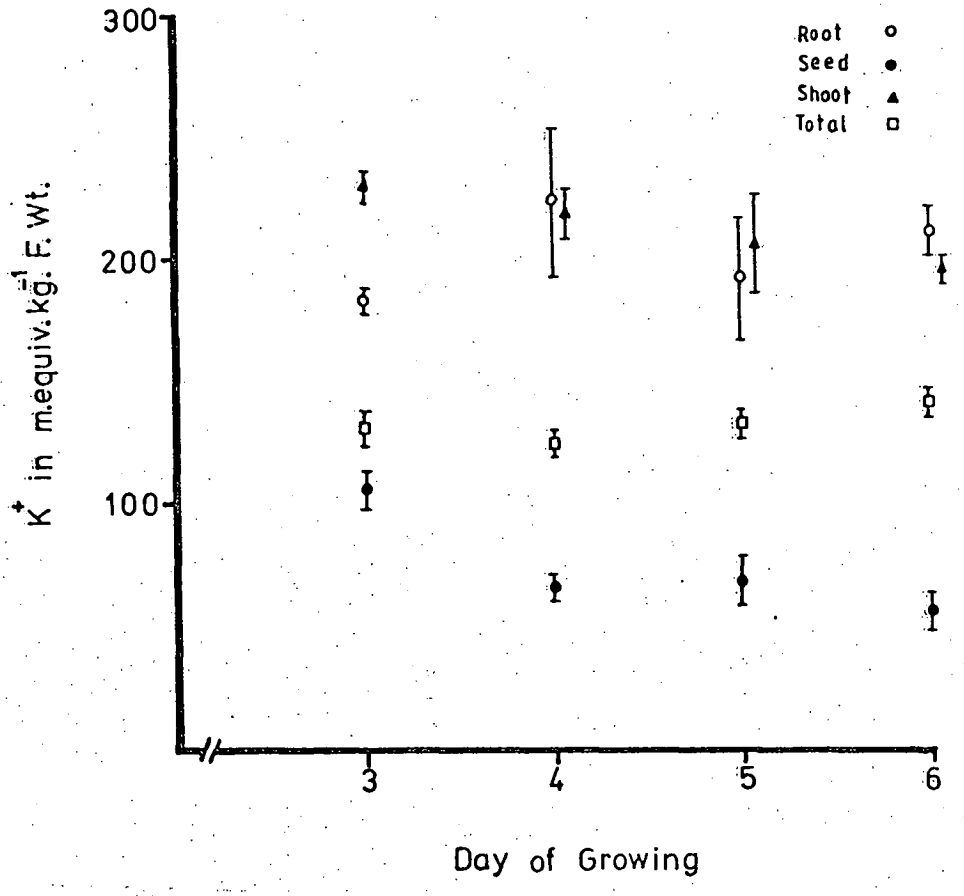


Fig. 3.6

Time course of K^+ content in the root (o), seed (●), shoot (▲) and the whole plant (□) from the 3th day to the 6th day of growing, in the dark. The limit is S.E. of the mean from 2 observations, 8 seedlings in total. Note that the content is relative to the fresh weight of the tissue used.

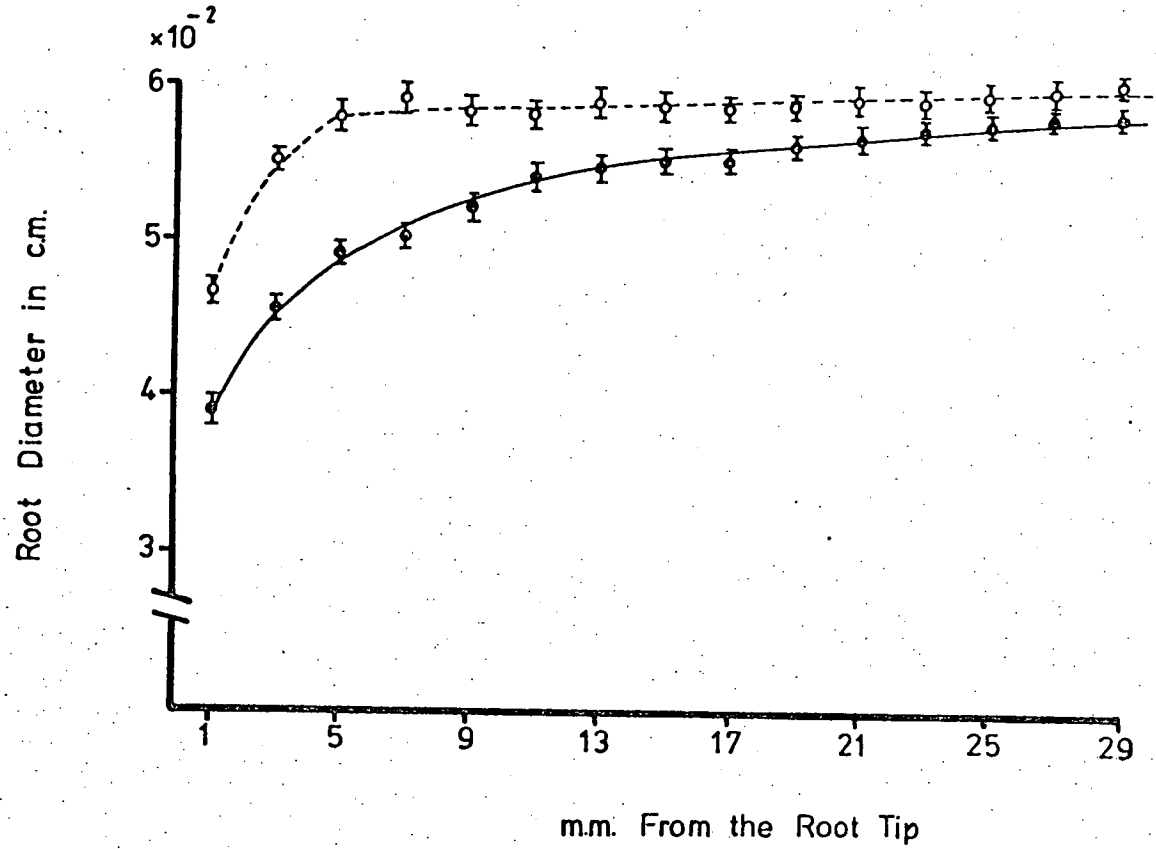


Fig. 3.7

Showing the change in diameter of the root along 30 mm from the tip from 4 days (o) to 6 days (●) of growing in the dark. The limit is the standard deviation of 20 roots.

by

$$\phi = (\partial q/\partial t) + (\partial q/\partial x)\, V \qquad (3\text{-}1)$$

where $q = c \cdot l \cdot a$

q is the average content in moles per cortical cell length, c; the average ion concentration in moles.kg^{-1}, l; the average cortical cell length of the root segment, a; the mass per unit length of the root, x; the interested position from the root tip at the beginning of the experiment and V; the rate of elongation of the root which can be obtained from section 3.4.1.

The mass per unit length (a) of the root was estimated by measuring the root diameter, as shown in Fig. 3.7, and assuming root density equal to water density. The measurement was made by placing the root on a glass slide with a cover slip on top, and viewing it through a microscope at 100x magnification. The concentration (c) of ions in each segment can be obtained from Fig. 3.5, after taking the mass into account. Utilising information of average cortical cell length (l), the values of q can be determined. Fig. 3.8 shows the content per cortical cell length plotted against the distance from the tip.

To calculate ϕ of K^+ in the cells of a mature region between 10-20 mm from the tip, the change of ion content at the middle of the region (at 15 mm) is considered. The content in this region decreases from the 4th day to the 6th day of growing at a rate of 57.9×10^{-13} moles.hr^{-1} $(\partial q/\partial t)$. The value of $(\partial q/\partial x)V$ which is averaged between two plant ages increases at a rate of 10.9×10^{-12} moles.hr^{-1}. Utilising the equation (3-1), the rates of K^+ accumulation per 10 mm root segment basis are 0.59×10^{-9} moles.hr^{-1}. Taking the average fresh weight of the tissue into account, the rate of accumulation in the mature cortical cells is about 0.5 m.equiv.kg^{-1}.hr^{-1}.

A similar method was used to estimate the accumulation rate of Cl^- ions. Since q hardly changes within 48 hrs, the rate is determined by the second term of the equation (3-1) alone. It is 0.44×10^{-9} moles.hr^{-1}, or 0.4 m.equiv.kg^{-1}.hr^{-1}, slightly smaller than that for K^+.

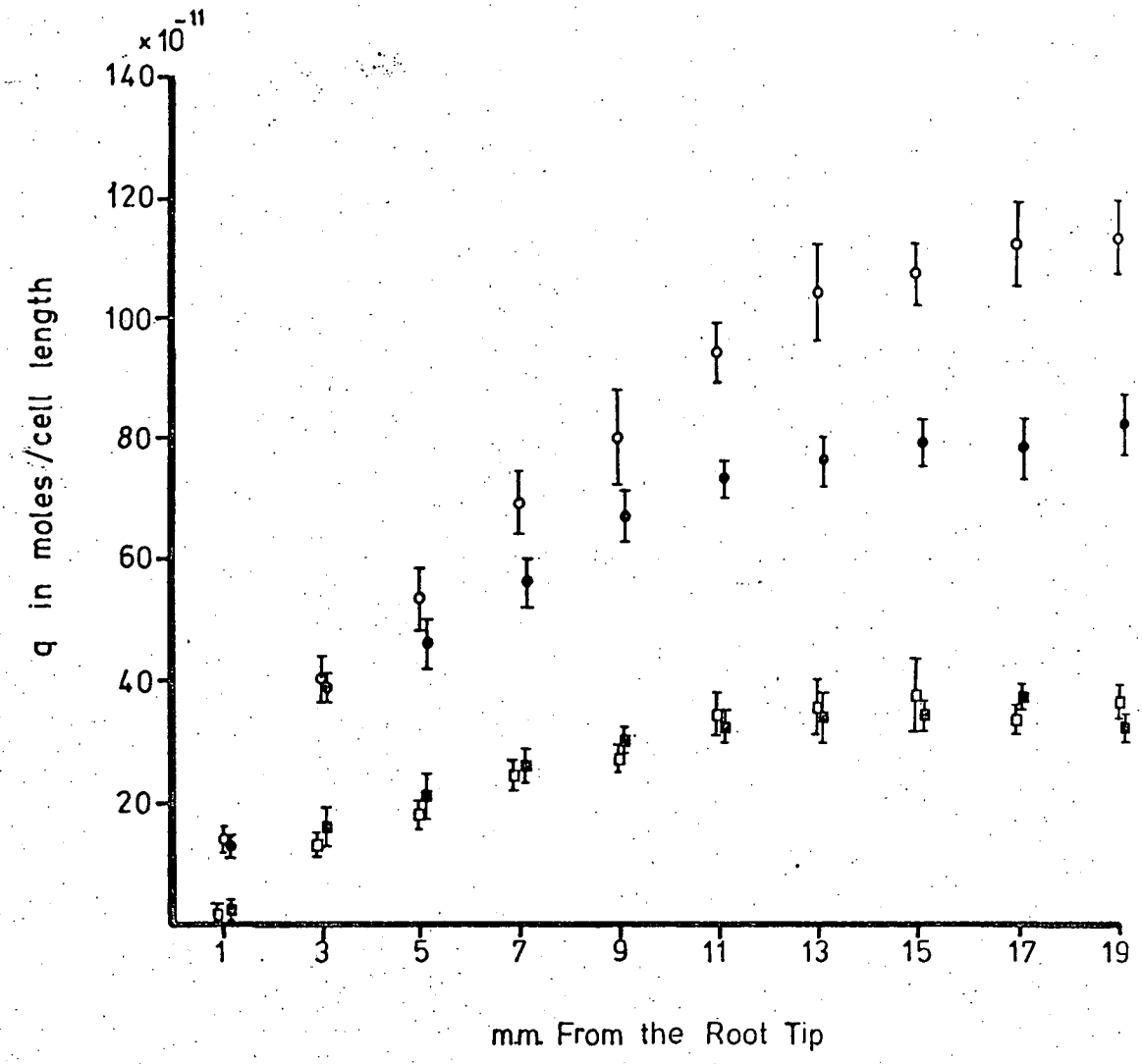


Fig. 3.8

Showing the content of K^+ (o,●) and Cl^- (□,■) ions per unit cortical cell length along a 20 mm root at the age of 4 days (open symbols) and 6 days (closed symbols), assuming density of root tissue is equal to water density and utilising the content per 2 mm segment from Fig. 3.5(a) and (b).

Within the limits of plant variation, it is concluded that the net accumulation rate of K^+ and Cl^- ions into the mature region of the root is negligible. This indicates that the root cells in this region are in a steady state. It should be noted that this finding is at variance with that in chapter 4 (section 4.4.1) in which roots were arranged horizontally. Under these conditions, a net loss of K^+ from the tissue was observed.

3.5 Discussion

As has been reported for most glycophyte plants, rice seedlings contain more K^+ than Cl^- or Na^+. The order of content among these 4 ion species is $K^+ > Cl^- > Mg^{+2} > Na^+$. The smaller amount of Cl^- than K^+ was also found in rice by Yoshida (1981).

At comparable distances from the tip, it was found in the present study that K^+ content was smaller at 6 days than at 4 days and this was associated with the smaller size of the root. This could be because high level of K^+ in roots of young seedlings may come from reserves in the seed, which is the main source of ions at an early stage of growth. In the later stage, roots depend more on ions absorbed from the external source and the culture solution was possibly not concentrated enough for the plants to keep up with ion requirements. To test this, seedlings were grown in a 10x concentration of the 1x solution and this is referred to as a 10x solution. Ion contents in the mature root region between 10-20 mm from the tip were analysed and compared with those obtained from roots grown in the 1x solution. It was found that the fall of K^+ content in the new root cells is reduced from 25%, taking between 11-19 mm from the tip, in the 1x solution to about 8% in the 10x solution. Change in the concentration to 10 times greater does not seem to have a marked effect on other ions.

On the structure of the root, it was found that the meristematic region of the root extends not more than 5 mm from the tip. Cell extension ceases after about 10 mm from the tip and beyond this distance cells are regarded as mature. In the 10-20 mm region, the xylem vessels occupy about 1.3% of the tissue volume (Plate 3.1). This is in the range of 1%-1.5% of the tissue volume as suggested by Clarkson et al. (1984). The finding of lateral root eruption from the region beyond 25 mm from the tip makes this region of the root more complex for ion flux measurements, if a model as shown in chapter 2 (Fig. 2.1) is to be utilised.

The observation of large spaces within the cortex of the root between 25-30 mm from the tip (section 3.4.2)

may be the initial stage of the larger scale air space development found in older rice roots (Hoshikawa 1975) and in anaerobically grown corn roots (Drew et al. 1980). Since rice seedlings were grown under a continuous aeration, the formation of these spaces agrees with the observation by Luxmoore and Stolzy (1969) in view that they do not depend on the oxygen concentration of the root medium for rice. As suggested by Yoshida (1981), they provide an efficient air passage from shoots to roots to allow respiration providing energy for ion uptake and cell division at the tip.

For anaerobically grown corn roots, Drew et al. (1980) suggested that these large spaces occur together with the collapse of certain cortical cells and that the wall residues are able to conduct ions from the outer cell layers to the xylem. Whether or not this is the case in rice roots, it is envisaged that the ability to absorb ions into this region of the root would be affected by the presence of the spaces to some extent and that the eruption of lateral roots occurs as a consequence. A reason supporting this is that if direct ion absorption into the cortical cells from the apoplast (Pitman 1977) still takes place in this region, the collapse of the cortical cells when the air spaces are formed would reduce the absorption surface area. Moreover, the development of a hypodermal casparian band in the mature region of the root is possible, as has been observed in corn and onion roots (Peterson and Perumalla 1984), which could reduce ion permeability of the root. However, the effect of the air space development on ion uptake and whether such hypodermal casparian band is also developed in the mature region of rice roots remain to be investigated.

Chapter 4

Potassium Kinetics in Intact Roots

4.1 Introduction

As with other plant cells, transport of ions in root cells involves sequential flow across the plasmalemma and the tonoplast. The evidence for this was observed from washout experiments after loading the tissue in a labelled solution for a period of time (Pitman 1963, Pallaghy and Scott 1969, Cram 1973, Erlandson 1979, Jeschke 1977, Jeschke and Jambor 1981, and Behl and Jeschke 1982). Consequently, a three compartmental model which includes the free space, the cytoplasm and the vacuole, is used with the addition of the xylem compartment when transport of ions across the root is studied. However, some workers observed that under certain conditions ion efflux from the labelled tissue was discontinuous (Pallaghy et al. 1970, Erlandson 1979, Jensen and Kylin 1980, and Behl and Jeschke 1982). The cause of the discontinuity was in dispute. The possibility of a fourth compartment residing in the cytoplasm as proposed by Pallaghy et al. (1970) was supported by Bange (1977), but disagreed with by Behl and Jeschke (1982).

Among the three most common ion species, K^+, Na^+ and Cl^-, whose movements across the roots are studied, K^+ is the most greatly accumulated ion in plant tissues. K^+ transport in root tissue is related to an important enzyme, ATPase, at the plasmalemma (Cheeseman et al. 1980). By using an ATPase inhibitor, a reduction of K^+ influx (Cheeseman et at. 1978) and H^+ efflux (Marre et al. 1974) was associated with a reduction of cell enlargement indicating the importance of K^+ in regulating the internal function of plants. Yet the mechanism of transport for this ion species is not fully understood. Part of the problem is probably that most studies were made in excised tissues, and in some investigations irregularities of ion elution from plant roots were found (Behl and Jeschke 1982). So far, there has been only one report of K^+ compartmental fluxes in intact roots of barley by Jeschke (1982), but in that study the root tip was discarded due to complications caused by the inhomogeneity of the tissue. No study appears to have been made previously in a

portion of uniform cells of intact roots.

In a number of previous studies on plant tissues, ^{86}Rb was used as a tracer for K^+ ions (Cheeseman and Hanson 1979, Cheeseman et al. 1980, Gronewald and Hanson 1980, Jensen and Petterson 1980, and Nissen 1980). The longer half-life of ^{86}Rb (18.7 days) than ^{42}K (12.45 hours) makes it easier to handle the experiments. The use of this isotope, however, requires justification of its suitability. The suitability of ^{86}Rb for tracing the movement of K^+ ions has been investigated previously by a number of authors (Maas and Leggett 1968, Läuchli and Epstein 1970, Marchner and Schimansky 1971, Jensen and Kylin 1980, and Behl and Jeschke 1982). Experimental results showed that the difference in ion absorption into roots between these cations was related to the presence or absence of Ca^{+2} ions in the medium (Läuchli and Epstein 1970), and to the concentration of nutrient solutions used (Jensen and Kylin 1980). Work by Jensen and Kylin (1980), using solutions containing Ca^{+2}, showed that the uptake of Rb^+ differed from that of K^+ ions and the difference varied according to plant species. They also suggested that the discrimination site for these cations was at the tonoplast. Alternatively, Behl and Jeschke (1982) suggested the site was in the stele.

In connection with ion transport across the root, the appearance of plasmodesmata in both radial and longitudinal directions of root cell walls (Vakhmistrov et al. 1972) leads to the possibility of longitudinal exchange of ions between neighbouring cells in the root cortex, after they enter the symplast. The experiments of Behl and Jeschke (1982) suggest that the transport between non-vacuolated and differentiated cells may be substantial. In the simple Pitman model of ion transport, this exchange (which will be referred to as longitudinal transport) is assumed to be relatively small compared to that in the radial direction into the xylem. The validity of this assumption needs to be investigated.

The scope of the work in this chapter is the investigation of ionic fluxes in a portion of roots between 10-20 mm from the tip. Cortical cells in this region of the root was found to be relatively uniform (chapter 3). Moreover, this region

of the root is ideal for the study, since it is free from lateral roots. Although the preliminary studies described in chapter 3 showed that K^+ accumulation into mature cells in this region was close to zero, this is true for roots growing vertically. Since the method of flux measurement was designed in such a way that plants were treated horizontally during experimentation, it was necessary to test whether the zero accumulation still applies for roots arranged horizontally (see section 4.4.1).

In most experiments ^{86}Rb was used as a tracer for K^+ movements, and in some experiments the root tissue was labelled with both ^{86}Rb and ^{42}K isotopes so that the suitability of ^{86}Rb could be tested.

Unlike excised tissues, the amount of ions found in the medium during washing out of intact roots represents only a fraction of the total loss from the labelled portion. Since the rest is translocated to the shoot, it is necessary to devise experiments which allow the total loss to be determined from the amount observed in the washout solution (section 4.5.2).

An attempt was made to measure ion efflux from the xylem to the surrounding tissue. The results indicate that it is probable that reabsorption of ions from the xylem does occur.

4.2 Experimental Materials

4.2.1 Preparation of rice seedlings

The method of growing rice was the same as described in chapter 3, except they were exposed to 12 hr light and 12 hr dark on the fourth day. The light intensity was about 1,000 lux, a combination of fluorescent and incandescent. The age of plants was 5 days in all experiments. The composition of the culture solution was that of the 1x solution (see section 3.2, chapter 3), which was also used in uptake and washout experiments except when the solution was augmented with ^{86}Rb or ^{42}K.

4.2.2 A K^+-free solution

Some of the experiments in this chapter required a solution without K^+ ions. It was prepared by substituting RbCl for KCl in the normal 1x solution at the same 1.0 mM concentration. The pH value of this solution was 5.6, the same as that of the 1x solution.

4.2.3 Isotope labelled solutions

^{86}Rb and ^{42}K were supplied by Amersham International, England and the Australian Atomic Energy Commission, N.S.W. respectively in the form of Cl^- salt solutions. The activities of the solutions used for uptake and washout studies were 2 μCi/ml and 20 μCi/ml , respectively.

The term "uptake" used in this study and also in chapter 5 refers to ion accumulation in the root and transport into the shoot, while "washout" refers to the washing of a labelled tissue in a non-labelled solution.

4.3 Experimental methods

4.3.1 Measurements of tracer efflux from a labelled portion of the root

All of the measurements in this chapter were made using an apparatus shown in Fig. 4.1. Roots were blotted gently and arranged horizontally in the apparatus. A water repellent grease was used to seal the partition between compartments. The root tissue in the middle chamber, between 10-20 mm from the tip, was labelled for 2-24 hours with a 20μCi/ml labelled solution. The side chambers were filled with a 1x solution. The levels of the solutions in these chambers were such that that in the middle one was lower than the others, so that any occurrence of leakage between chambers could be observed. The ratio of the labelled solution to tissue volume was about 200:1, while that of the non-labelled tissues were 400:1 and 1700:1 for the tip and the basal part of the root, respectively. At the end of loading and if there was no sign of leakage, the labelled solution was removed with a hypodermic needle. To wash away the remaining isotope in the chamber, an amount of a non-labelled solution (1x solution) was injected into the chamber around the wall, without disturbing the labelled root tissue. This solution was removed within 20 seconds.

Washing out commenced when the labelled tissue in the middle chamber was submerged with a 1x solution. The solution was collected with a hypodermic needle at the end of different periods of time. Washout times were gradually increased from 10 min to 1 hour. After 5 hrs of elution had passed, collection was made at longer periods. At the completion of elution (30-45 hrs), solutions in the middle chamber and in the chamber above the washed tissue were removed. The root was cut at the end of the middle chamber to stop ion transport into the shoot. The rest of the root, in the middle and the tip chamber, was blotted and excised to separate the washed tissue from the tip region. The excess length of 2 mm root at both ends of the tissue which was originally confined in between compartment walls was discarded. The washed tissue was blotted and weighed before being crushed,

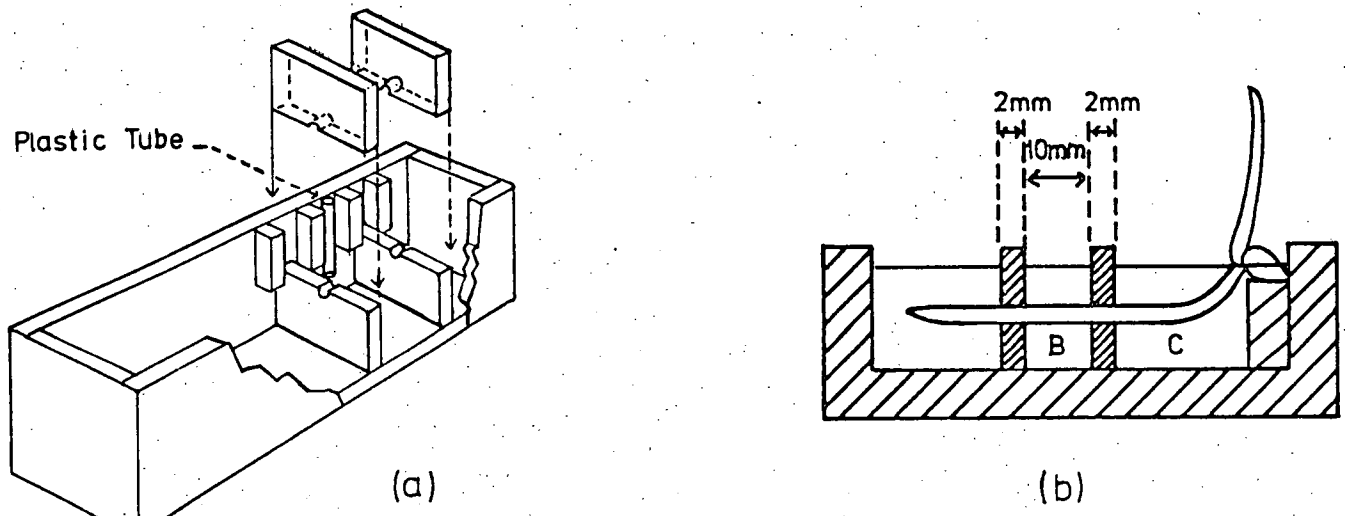


Fig. 4.1

An apparatus used for flux studies in a portion of an intact root. It was 80x15x20 mm, made from perspex. The apparatus was divided into 3 chambers; the middle chamber with 10 mm in length and side chambers.

(a) Showing a three dimentional drawing of the apparatus. A plastic tubes (2 mm , in diameter) was attached to each wall of the middle chamber; one for solution inlet and the other (opposite to the one shown in the figure) for aeration. In washout studies, samples were collected via the solution inlet tube by using a hypodermic needle to avoid any disturbance to the root (Fig. b).

(b) Showing a side view of plant root arranged in the apparatus during experimentation. A portion between 10-20 mm from the tip was in the middle chamber.

dried, and counted. In some experiments, the non-labelled tissues in both side chambers were also brought to count, so that their activities could be compared with the labelled one. The seeds were husked before being counted.

Each sample was counted in a small aluminium planchette (2 cm in diameter). After the sample was dried slowly on a hot plate, it was transferred to an automatic sample changing machine (see Plate 4-1) which could handle 12 samples at a time. A Geiger Müller counter (Phillips 18504) was mounted above the sample plate. When root tissues were to be counted, they were crushed and evaporated in the planchette.

It should be noted that in some experiments leakage occurred between the compartments and this caused irregularities in the washout graphs. Such cases were rejected. It is essential to mention that in some experiments, washout was followed for 30-40 hrs. By the end of this time, the tip portion of the root had elongated from 10 mm to about 45 mm and lateral roots had appeared in the region of the root under study. Their appearance, however, did not affect the linearity of the graph greatly, and in any case the long-term slope of the washout graph (k_L-section 4.5.2) was usually obtained much earlier (about 5- 15 hrs).

Due to the finding of the discrimination against Rb^+ in some plant species, some experiments in this section were carried out using both ^{86}Rb and ^{42}K tracers in the same labelled solution. The activities per ml of the labelled solution was 10 μCi for ^{42}K and 5 μCi for ^{86}Rb, respectively. If ^{86}Rb is a suitable tracer for K^+, the amount washed out into the bathing medium and that remaining in the tissue at the end of washing should be independent of which isotope is used. Due to the shorter half-life of ^{42}K, all activities in the tissue were corrected to the time when the specific activity of the medium was determined and adjusted to the standard value of 0.90×10^{14} cph.mole^{-1} of K^+ in the solution.

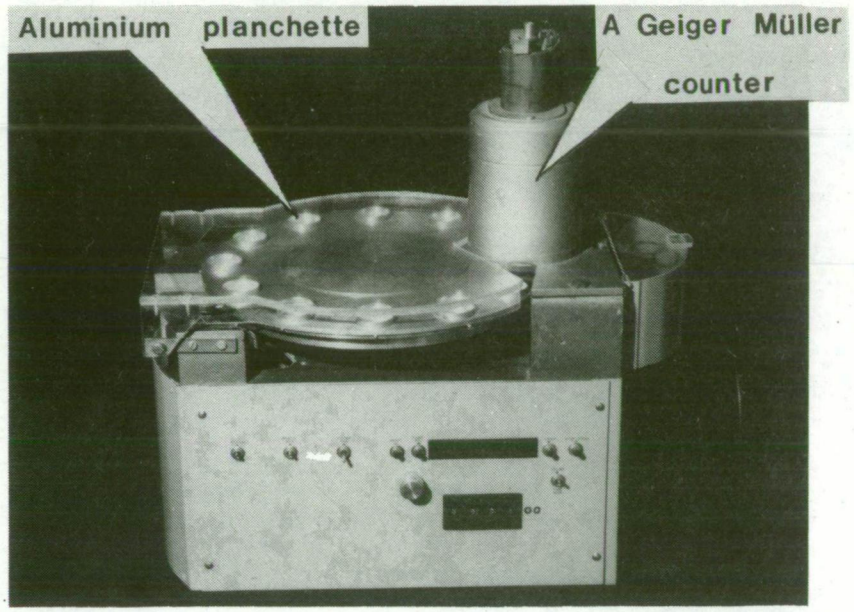


Plate 4-1. A Sample Counting Machine

A Geiger Müller counter is mounted above an automatic sample changer. Each sample was placed in an aluminium planchette and counted twice.

4.3.2 The determination of K^+ content in plant tissues

After plants were grown horizontally for various periods of time, they were washed in a K^+-free solution for 30 min before being blotted and excised. The seed was separated from the shoot and the root. In some experiments (section 4.4.1) when K^+ content in a mature portion of the root which was originally between 10-20 mm from the tip at 0 hour was of interest, that portion of the root was separated from the rest of the root. K^+ ions were analysed using the method described in chapter 3, section 3.3.2.

4.3.3 Measurements of ion uptake into a portion of roots by using a tracer

After seedlings were arranged in the apparatus, the middle chamber was filled with a 2 μCi/ml labelled solution and the others with a 1x solution. At the end of loading, root tissues were separated into portions comprising 0-10 mm, 10-20 mm, and 20 mm to the base of the root. Activity in the latter was combined with that of the seed and the shoot. The mass of the root between 10-20 mm was recorded.

Experiments in sections 4.3.1 to 4.3.3 were carried out under the same conditions as growth, at 25° C. Aeration was supplied only in the middle chamber throughout the experiments. Each observation contained 4 seedlings. A neutral red solution was dropped into the middle chamber at about 10-15 min before each experiment ended to distinguish the observed portion of the root from the rest. This was also used as an indication for solution leakage between chambers for a short-term loading period.

4.3.4 Measurements of the speed of flow in the xylem

Roots of 5 day old seedlings were blotted and placed on a glass slide over a 60-mm scale, as shown in Fig. 4.2. A chamber of 10 mm long was constructed using a water repellant grease. The root was arranged in such a way that the portion

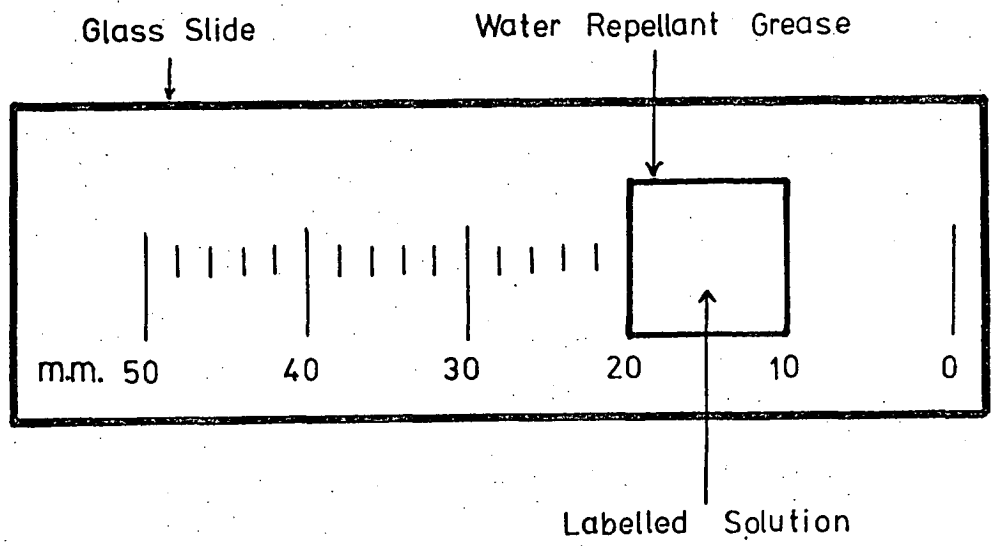


Fig. 4.2

Showing a schematic drawing of an apparatus used for measurements of the speed of flow in the xylem. The apparatus was made from a glass slide with scales. The window for isotope labelling was constructed with a water repellant grease, 10 mm in length. During experimentation, a plant root was placed on the slide in such a way that the root tip was at 0 mm. At the end of labelling, the root tissue beyond 20 mm from the tip was excised into 2 mm segments.

between 10-20 mm from the tip was confined in the chamber. A labelled solution of 40 μCi/ml activity was used, so that the movement of ions in the xylem could be detected easily. Times taken for tissue loading were 5 min and 10 min. The rest of the root was bathed with a 1x solution.

When loading ended, all solutions were removed and the basal part of the root was blotted with filter paper. Root excision was made above the labelled tissue into 2 mm segments until 7-10 segments were obtained. These segments were brought to count, as described in section 4.3.1. Time taken for the excision was between 0.5-1.0 min. To curtail further transport of the isotope front in the xylem during excision, one seedling was studied at a time.

To detect for any leakage from the labelled chamber, the blotting paper was also counted. If the activity found was greater than what was observed from the segment next to the chamber, the experiment was rejected.

4.3.5 Measurements of ion efflux from the xylem vessels by using ^{86}Rb as a tracer

The measurement in this section was based on the assumption that tracer found in tissues outside the loading region had reached this tissue either by longitudinal transport between cortical cells via the symplast and possibly the apoplast, or by re-absorption from the xylem. In the former case, it was assumed that the transport was bidirectional, whereas xylem transport was assumed to be upward only. The difference between the amount of tracer found in the non-labelled tissues above and below the labelled portion of the root was taken to be the amount reabsorbed from the xylem. The validity of this assumption will be discussed later.

The same apparatus as in the above section was used in this measurement, except that at both ends of the middle chamber was constructed a 5 mm guard chamber. A 2 μCi/ml labelled solution was used in the middle chamber. The labelled portion of the root was between 15-25 mm from the tip. At the end of loading, the non-labelled tissues beyond the guard chambers were

blotted and excised at 10 mm and 30 mm from the tip. A further excision of the root was made at 40 mm from the tip. These 10 mm root segments, one between 0-10 mm and the other between 30-40 mm from the tip, were washed in a 1x solution for 1 hour before being counted. This period of time is long enough to wash away the tracer in the xylem and most of that in the cytoplasm (see Fig. 4.6).

It should be noted that experiments in section 4.3.4 and 4.3.5 were carried out at room temperature, about 20° C. Aeration in the middle chamber of the experiment in section 4.3.5 was provided occasionally. As in section 4.3.1, leakage between chambers during long-term loading periods (section 4.3.5) was observed by the change of the solution levels.

To estimate the specific activity, an amount of the labelled solution was diluted to a known volume and 10^{-2} ml of this was dried and counted. For 2 μCi/ml solution, no dilution was made. The specific activity is calculated from the ratio of the solution activity to the number of moles of K^+ ions in the solution. In all experiments with tracers, self absorption was taken into account only when the tissue above the labelled portion of the root was counted. This was determined by recording the activity of the labelled solution before and after placing the tissue in a planchette (see section 4.3.1). It was found that self absorption in 10 mm root tissue was small. All activities reported in following sections were corrected for the background activity.

4.4 Results

4.4.1 K^+ uptake into roots : A comparison between vertically and horizontally grown plants

Since experiments in this chapter were to be carried out using plants arranged horizontally, it was therefore necessary to study how the orientation affected K^+ uptake. The study was made by separating 5-day old seedlings into two groups; one was left in the growing system after changing the culture solution to a fresh one, and the other was arranged in the apparatus. To treat the latter identically to plants used for washing out, all three chambers were filled with a 1x solution and a water repellent grease was used in between the compartments. Root and shoot length were recorded before and after the seedlings were grown in the apparatus. To avoid undue change in concentration, the solution was changed every 12 hrs. A drop of neutral red solution was added to the middle chamber about 5-10 min before the harvesting time. This was done so that the portion of roots could be distinguished during root excision. At certain time intervals, they were harvested and washed in a K^+-free solution for 30 min by submerging the whole plant in the solution. The portion of root in the middle chamber was separated from the others and the seeds were husked. All tissues were blotted and weighed.

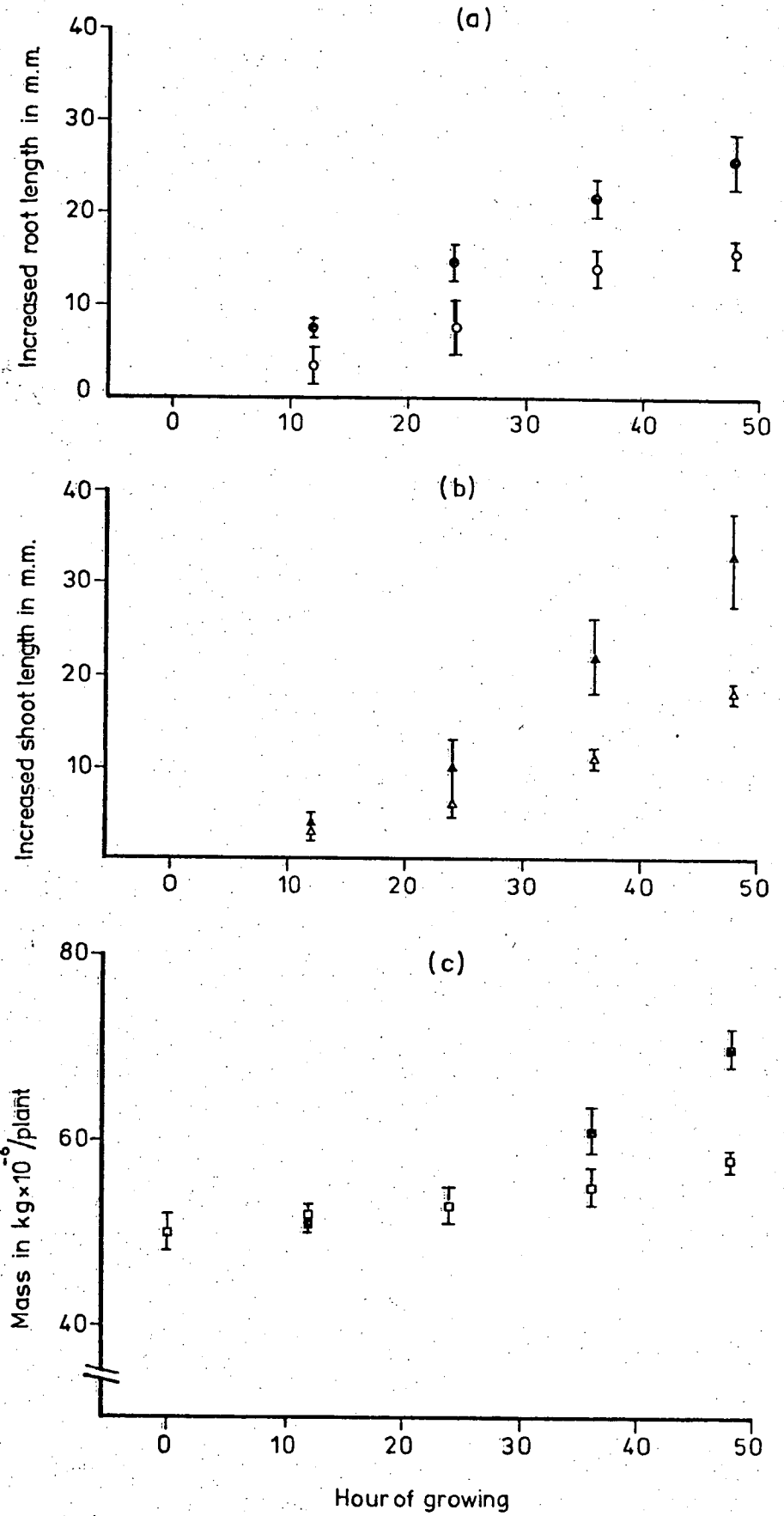
For vertically grown plants, length of roots and shoots were recorded at the beginning of the experiment and also at the same interval when horizontally grown plants were harvested. They were washed in the K^+-free solution for 30 min. Based on information of root growth, a portion which was initially between 10-20 mm from the tip could be followed.

Fig. 4.3(a) and (b) compare the root and shoot growth of vertically and horizontally grown plants, relative to the length at 0 hr. 5 observations were made with a total of 20 plants. It was found that there was some difference in the growth between two groups of plants. After 12 hrs of treatment, roots of horizontally grown plants are longer than those of vertically grown ones. This is also true for shoot growth, except

Fig. 4.3

A comparison between growth of vertically (open symbols) and horizontally (closed symbols) grown plants, under a controlled 25° C temperature in the light. The increased length of roots and shoots were relative to that at 0 hour. The limit is the standard error of the mean values from 5 observations. Each data point is the average of 20 plants.

(a) root growth (○,●)
(b) shoot growth (△,▲)
and (c) total fresh weight (□,■).


(a)
Increased root length in m.m.
40
30
20
10
0
0
10
20
30
40
50
(b)
Increased shoot length in m.m.
40
30
20
10
0
10
20
30
40
50
(c)
Mass in kg×10⁻⁶/plant
80
60
40
0
10
20
30
40
50
Hour of growing

the significance appears after 24 hrs. When the total masses of the plant groups are compared after 24 hrs, in Fig. 4.3(c), that of horizontally grown plants is significantly greater. It was observed that adventitious roots erupted after about 24 hrs of horizontally growing and a greater number of such roots appeared in horizontally grown plants.

The amount of K^+ ions found in the whole plant, expressed in equiv.kg^{-1} fresh weight, is shown in Fig. 4.4(a). Despite the difference in the growth rates, the total amount per unit fresh weight of plants from both groups is similar. The consistency between the larger number of adventitious roots and the increasing growth rate of horizontally grown plants indicates some regulatory systems in plants for the mineral requirement for growth. The average rate of ion increase obtained from both plant groups is 0.80 m.equiv.$kg^{-1}.hr^{-1}$. When the amount in 10 mm portions of roots are compared, Fig. 4.4(b) shows that ion accumulation in the tissue for both plant groups appears in diurnal patterns with 12 hour period between two peaks. This pattern of uptake was also found in roots of Italian ryegrass for NO_3^- ions by Hansen (1980), which was concomitant with root respiration. Graphs also show a trend of fall in the accumulation in horizontally grown roots with age. The fairly constant level of ion accumulation in the tissue of vertically grown plants agrees well with the result in chapter 3 (section 3.4.4).

To confirm that there is a net loss of ions from the 10-20 mm portion of horizontally grown roots, the same experiments were repeated in 4 replicates, and the results were combined with the above one. The time course of K^+ accumulation in the tissue is shown in Fig. 4.5(a). As is shown, the accumulation decreass significantly between 24 hrs and 48 hrs. When the difference in the accumulation between these time intervals $[K^+]_t$ and the 0 hour one $[K^+]_o$ are plotted against time, the graph (Fig. 4.5b) shows that there is a slight decrease in the accumulation during the first 24 hr period, though not significant. At 48 hrs, however, a marked decrease occurs and its appearance is in a non-linear fashion compared to the 24 hr one. It is worth pointing out that the period between 24-48 hrs coincides with the evidence of the increasing shoot growth rate.

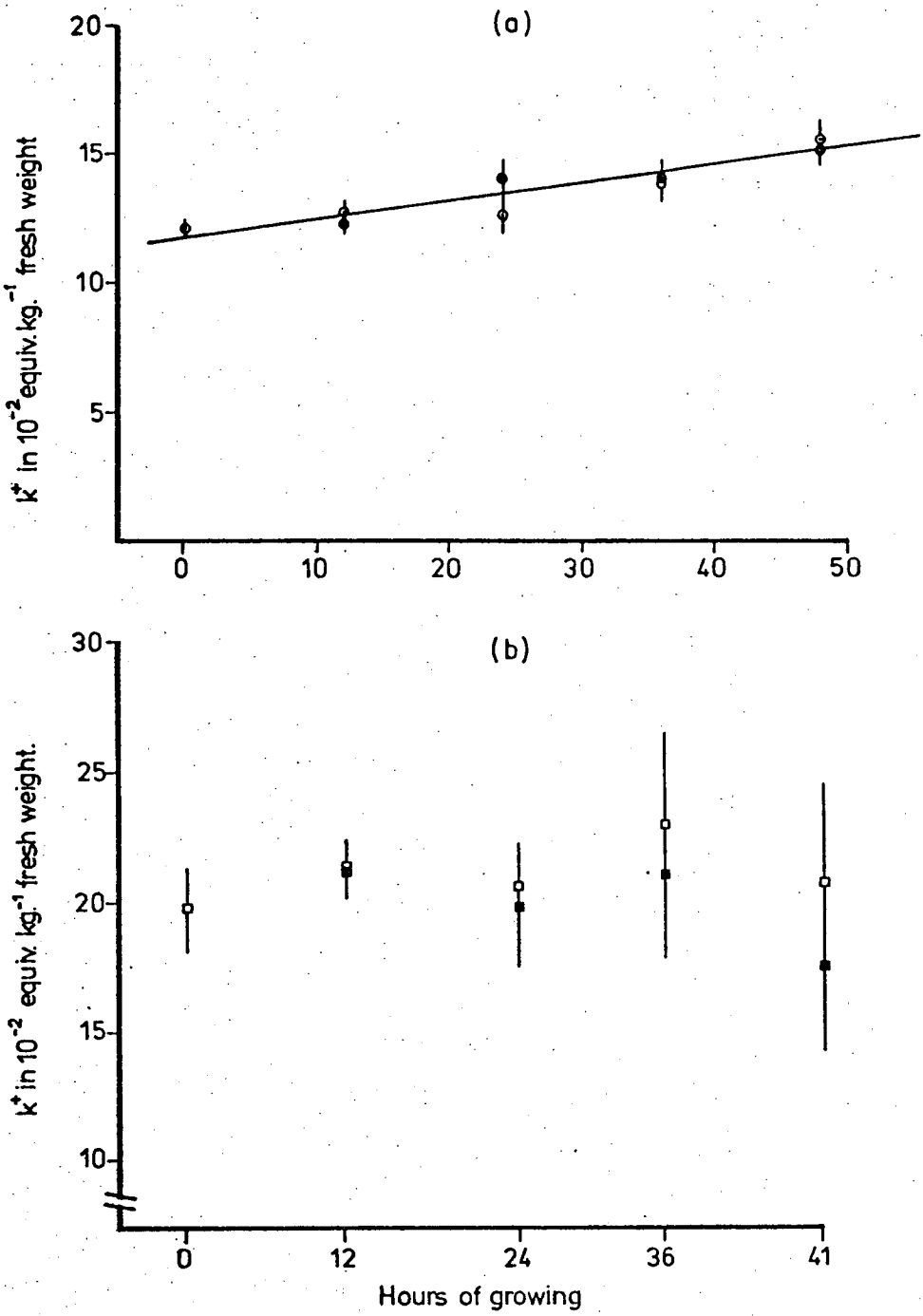


Fig. 4.4.

A comparison of K^+ content between plants grown vertically (open symbols) and horizontally (closed symbols), expressed in m.equiv.kg^{-1} fresh weight. The limit is the standard error of the means from 5 observations.

(a) the content in the whole plant (o,●)

(b) the content in a portion of the root which was originally between 10-20 mm at the beginning of the experiment (□,■).

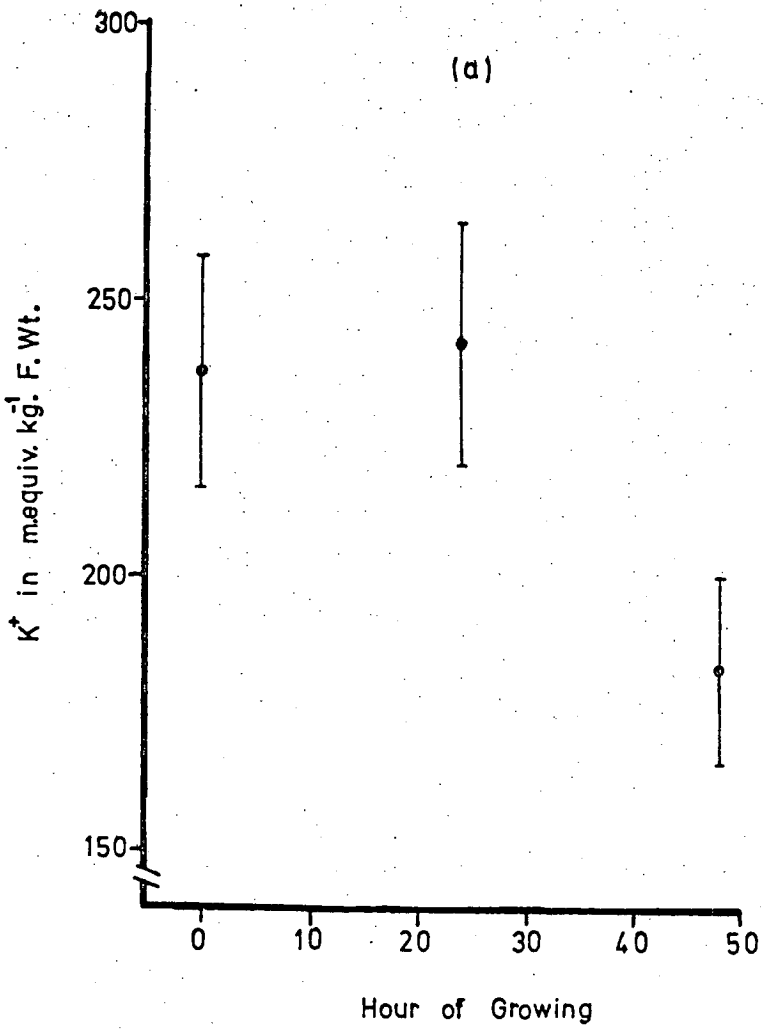
Fig. 4.5

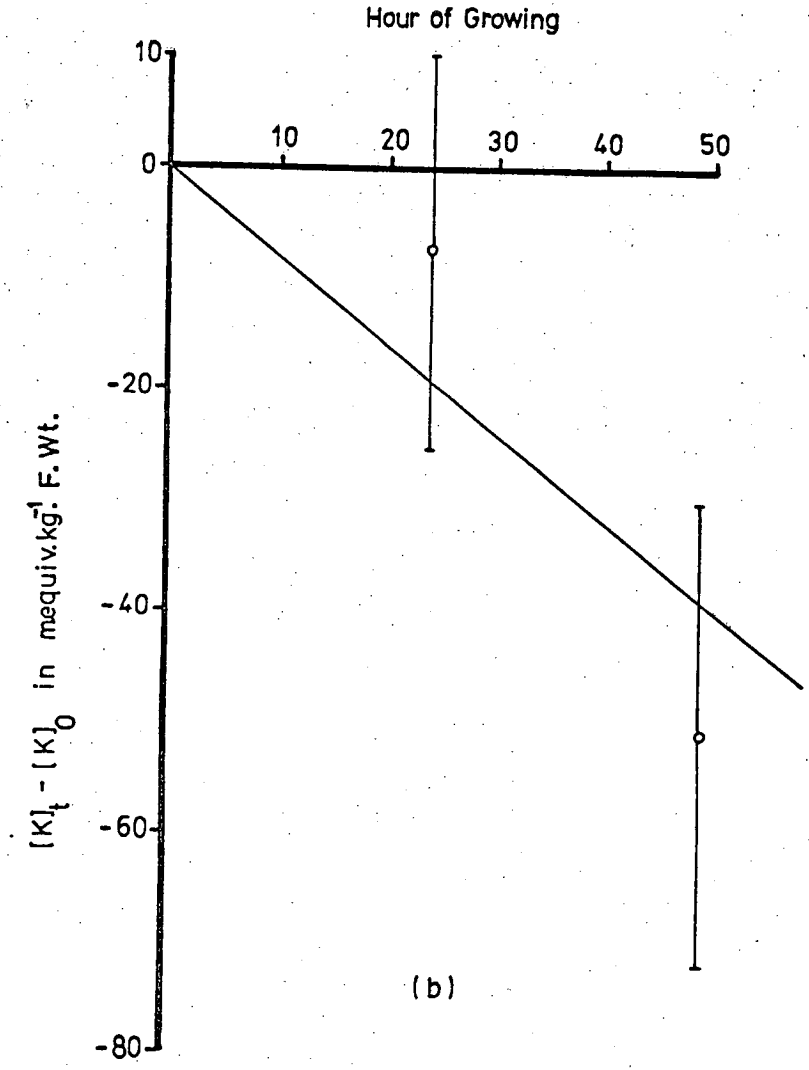
Showing the change in the amount of K^+ ions in a mature porion of the root after being grown horizontally in an apparatus shown in Fig. 4.1 for 24 hrs and 48 hrs, under 12 hr light 12 hr dark conditions at 25° C. The culture solution was a 1x solution (section 3.2). The portion of the root was initially between 10-20 mm from the tip at 0 hour and was furhter away from the tip at a rate of 0.7 $mm.hr^{-1}$ (see Fig. 4.3).

(a) showing time course of K^+ concentration in the tissue.

(b) showing the difference in the concentration of the tissue between 24 hrs or 48 hrs $[K^+]_t$ and o hour $[K^+]_o$.

The unit is expressed in relation to fresh weight of the root portion. The limit is the standard error of the mean of 9 observations. Each observation contained 4 roots.


(a)
300
250
200
150
0
10
20
30
40
50
K+ in mequiv. kg-1 F. Wt.
Hour of Growing


Hour of Growing
10
0
-20
-40
-60
-80
10
20
30
40
50
[K]t - [K]0 in mequiv.kg-1 F. Wt.
(b)

The considerable variability among plants, even those from the same batch, makes it very difficult to obtain an estimate of the rate of loss of K^+ from root tissue. The best estimate that could be obtained from those observations was 0.79 $m.equiv.kg^{-1}.hr^{-1}$. Most of this amount is likely to be transported into the shoots rather than eluted into the medium (see section 4.5.1), since the growth rate of these plants is greater than vertically grown ones.

Since there is a net loss of K^+ from the portion of the root under investigation, the exact solution of the flux equations (chapter 2) cannot be used in the analysis and it is necessary therefore to use the approximate solution. This is used in section 4.5.

4.4.2 The short-term characteristics of washout from a portion of an intact root

A portion of an intact root between 10-20 mm from the tip was bathed in a ^{86}Rb containing solution for a period time, followed by washing in a non-labelled solution for selected time intervals as described in section 4.3.1. At the end of washing, the amount of tracer found in the tissue was recorded as Y_r. As mentioned in chapter 2, the mathematical analysis requires that the relationship between the tracer in the tissue (Y) be determined as a function of time during washout. This can be obtained from the amount of tracer washed out into the bathing solution (Y′) if the tracer lost to the xylem during washout is taken into account. Fig. 4.6(a) shows a typical graph of Y′, in cph/root, plotted against time on a non-logarithmic scale. The initial portion of this graph (before the rate of increase in Y′ becomes steady) is the short-term loss to the bathing medium and includes loss from both the free space and the cytoplasm (Y_{fs}'), and when this component is plotted on a semi-logarithmic scale (Fig. 4.6b) gives the short-term rate constant (k_s) and Y_s' - the short-term loss to the bathing medium. Values of k_s and Y_s' obtained from the graphs are shown in Table 4.1(a). To convert the activity to $m.equiv.kg^{-1}$, the specific activity of the labelled solution and the tissue fresh weight were taken into

Fig. 4.6.

(a). Showing the short-term characteristics of ^{86}Rb elution from the labelled portion of roots into the medium (Y') with time. Y'_{fs} represents the amount appearing in the free space plus the short-term loss into the bathing medium.

(b) Time course of Y'_{fs} eluted into the medium from the labelled tissue. Y_f represents the amount of tracer in the free space at the end of loading, and Y'_s the short-term loss of the tracer into the medium. The slope is the rate of short-term exchange (k_s).

Data was from experiment # 3 of Table 4.1 (a). The standard specific activity of the labelled solution (s_o) was 0.9×10^{14} cph.mole^{-1} of K^+ .

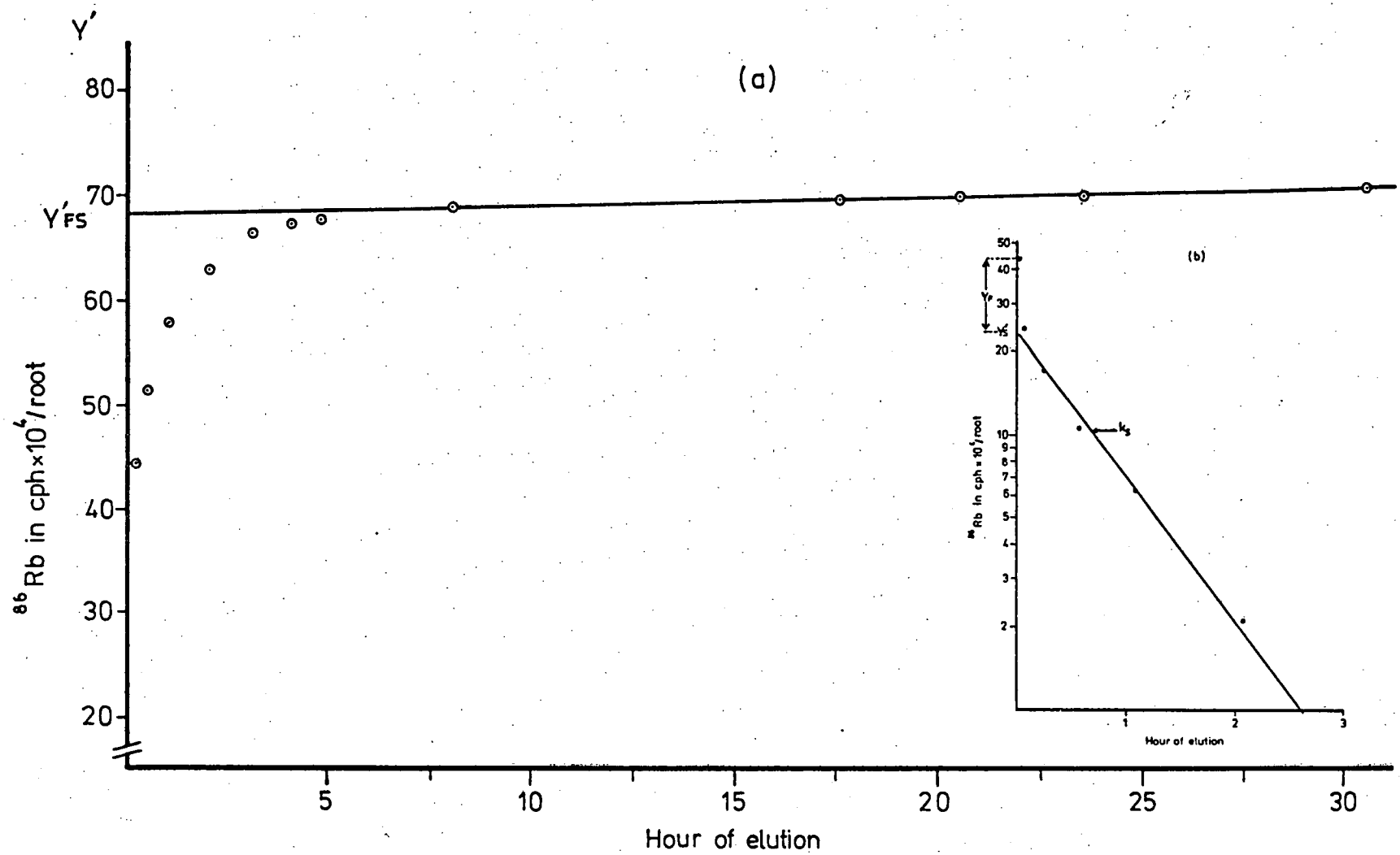

(a)
Y'
80
70
Y'FS
60
50
40
30
20
86Rb in cph×10⁴/root
5
10
15
20
25
30
Hour of elution
(b)
50
40
30
Y'
Y'S
20
10
9
8
7
6
5
4
3
2
86Rb in cph×10³/root
kS
1
2
3
Hour of elution

Table 4.1

Showing values of k_e, Y_e' and Y_r obtained from ^{86}Rb washout (a) and ^{42}K washout (b) from root segments.

T = loading time.

(a) ^{86}Rb washout

	Exp. no.	T hr	k_e hr^{-1}	Y_e' m.equiv.kg^{-1}	Y_r m.equiv.kg^{-1}
	1	2	.63	.55	1.40
	2		.75	.67	.12
*	3	5	1.20	1.94	.46
	4		.54	2.34	.39
	5		.97	1.92	.34
	6	5	.71	.16	.26
	7		.69	1.14	.17
	8		.84	.47	.60
	9		1.01	.75	.23
	10		.76	.17	.57
	11	24	.60	4.64	2.62
	12		1.22	7.88	4.03
	$\bar{X}$**		.81	1.01	.45
	±		±	±	±
	S.E.		.06	.25	.12

(b) ^{42}K washout

Exp. no.	T hr	k_e hr^{-1}	Y_e' m.equiv.kg^{-1}	Y_r m.equiv.kg^{-1}
1	5	.85	.49	.44
2		.66	1.20	.33
3		.72	.59	1.21
4		.96	.76	.91
5		.97	.76	1.03
$\bar{X}$		.83	.76	.78
±		±	±	±
S.E.		.06	.12	.17

* = data shown in Fig. 4.6

** = not included the 24 hours loading.

account.

The results show that k_e tends to vary according to plant samples. The values of Y_e' and Y_r increase with T. When studies from ^{86}Rb and ^{42}K are compared, the averaged k_e and Y_e' values are not significantly different. This suggests that there is no discrimination in the short-term characteristics of the tissue. The significance of this will be considered further in section 4.5.1.

It is important to note that the activity found in the tissue at the end of washing (Y_r) was greater when ^{42}K was used as a tracer than when ^{86}Rb was used.

In order to determine the distribution of tracer in and around the plant after various periods of loading, the following experiments were performed:

4.4.3 Determinations of the apparent influx (Y_{in}), longitudinal transport along the root (Y_t) and transport into the shoot (Y_x)

When a portion of the root is labelled, the amount of tracer ions found in the tissue above the 10-20 mm portion of the root at later times is assumed to come from transport in the xylem and from longitudinal transport along the symplast, and probably the apoplast, of cortical cells. It is also assumed that the amount found in the tissue below the labelled portion is from the longitudinal transport only. The unidirectional transport characteristics of xylem in this region of the root will be discussed later in chapter 8, section 8.6. During the experiment, some of the tracer which travelled to the non-labelled tissue in both side chambers could be eluted into the medium bathing them at a later stage. The following experiment was, therefore, carried out by taking the solution in the chambers into account. Only 1 ml of the solution in the upper chamber (i.e. above the labelled tissue) was dried and counted, after the total solution volume was recorded. Root excision was made as soon as the loading ended and after the removal of all solutions. The excision left an excess 2 mm length (the thickness of compartment wall) at each end of the labelled portion. Time

taken for washing the surplus isotope was 10 min. The extra length of tissue was discarded before the labelled tissue was weighed and counted.

The results from this experiment are shown in Fig. 4.7. The graph shows that tracer absorption into the labelled tissue (Y_{in}) increases at a fairly steady rate but there is a lag phase of transport into the shoot (Y_x) during the first hour of loading. Such a lag phase would be expected as the tracer equilibrates with the cytoplasm of the cortical cells. Y_x increases at a steady rate between 1-4 hrs and appears to be smaller than Y_{in} at any loading time, though not significantly so after 3 hrs. After 4 hrs of loading, there is a sign of decreasing rate of tracer build-up in the upper part of the plant, and this occurs at a time when there is an increase in the rate of build-up in the tip region (Y_t). This suggests that tracer that has moved to the shoot via the xylem has re-translocated to the root tip via the phloem. This additional pathway to the root tip is reasonable to account for the increased uptake. In addition, part of the increasing rate of Y_t could be due to direct transport into the phloem in the labelled region (Pitman, personal communication). The much smaller and steady rate of Y_t during the initial 3 hrs is likely to be due to longitudinal transport alone.

When both ^{42}K and ^{86}Rb are used together in the same tissue, graphs of tracers transported into the shoot in Fig. 4.8(a) show that Y_x for both tracers are similar for all period of time. This indicates no discrimination along the pathway from external solution to the xylem. When accumulation by the root tissue is considered, Fig. 4.8(b) shows that Y_{in} increases at a steady rate over a 5 hr period, and that of ^{42}K is greater than ^{86}Rb after the first hour of loading. During the initial 1 hr period, the two graphs do not diverge significantly. This may be due to a lack of discrimination in uptake into the cytoplasm. The difference becoming apparent only when there is significant accumulation in the vacuoles. Thus it appears that the tonoplast discriminates against Rb^+ in favour of K^+. Interestingly, the initial 1 hour period coincides with the delay of transport into the shoot, suggesting that the likely cause of the delay is the

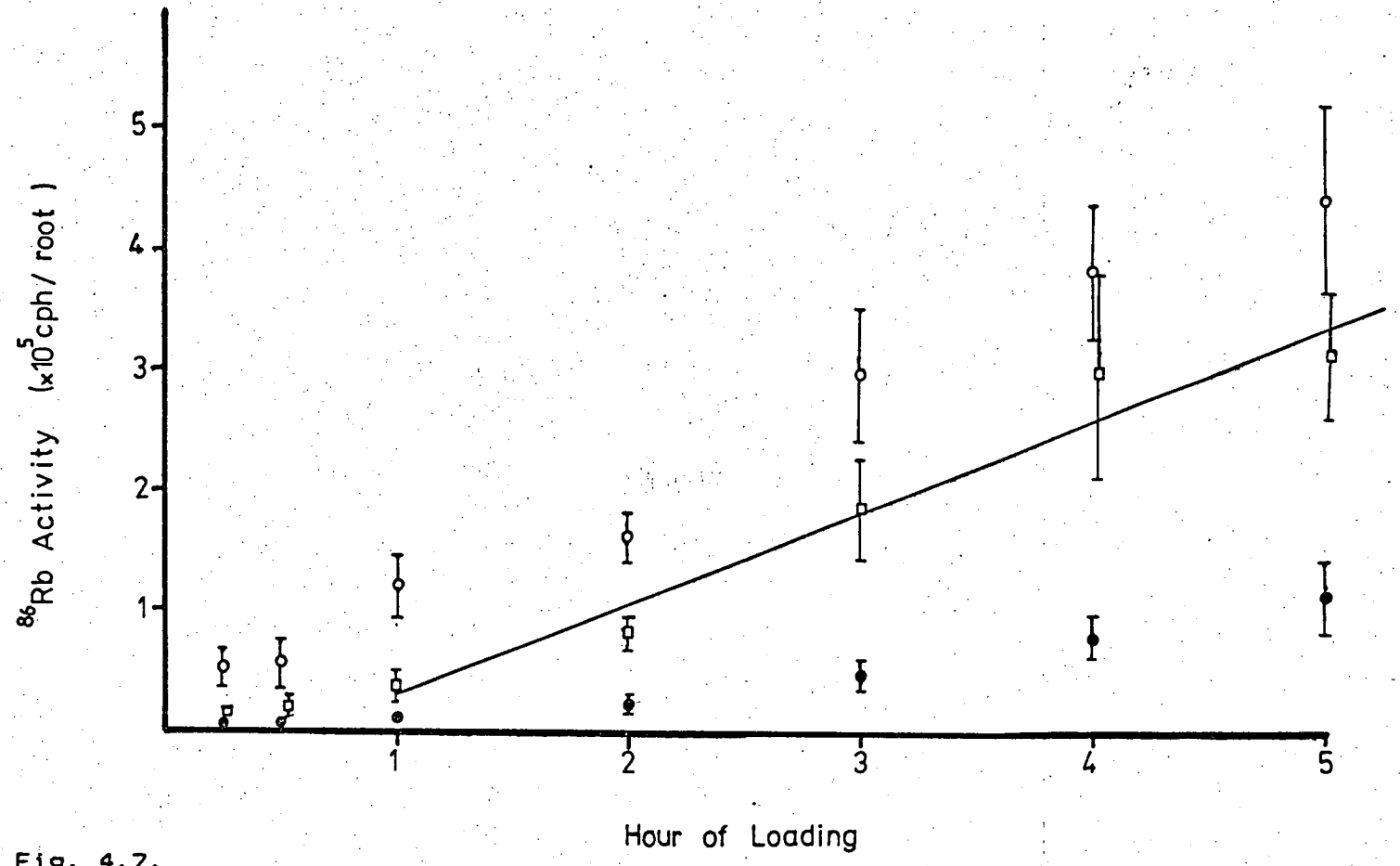


Fig. 4.7.

Time course of ^{86}Rb accumulation in the labelled portion of the root (o), transport into the shoot (□) and toward the non-labelled tip region (●). The limits are ± S.E. from 5 observations. The average mass of the labelled tissue was 1.26×10^{-4} kg./root.

Fig.4.8 .

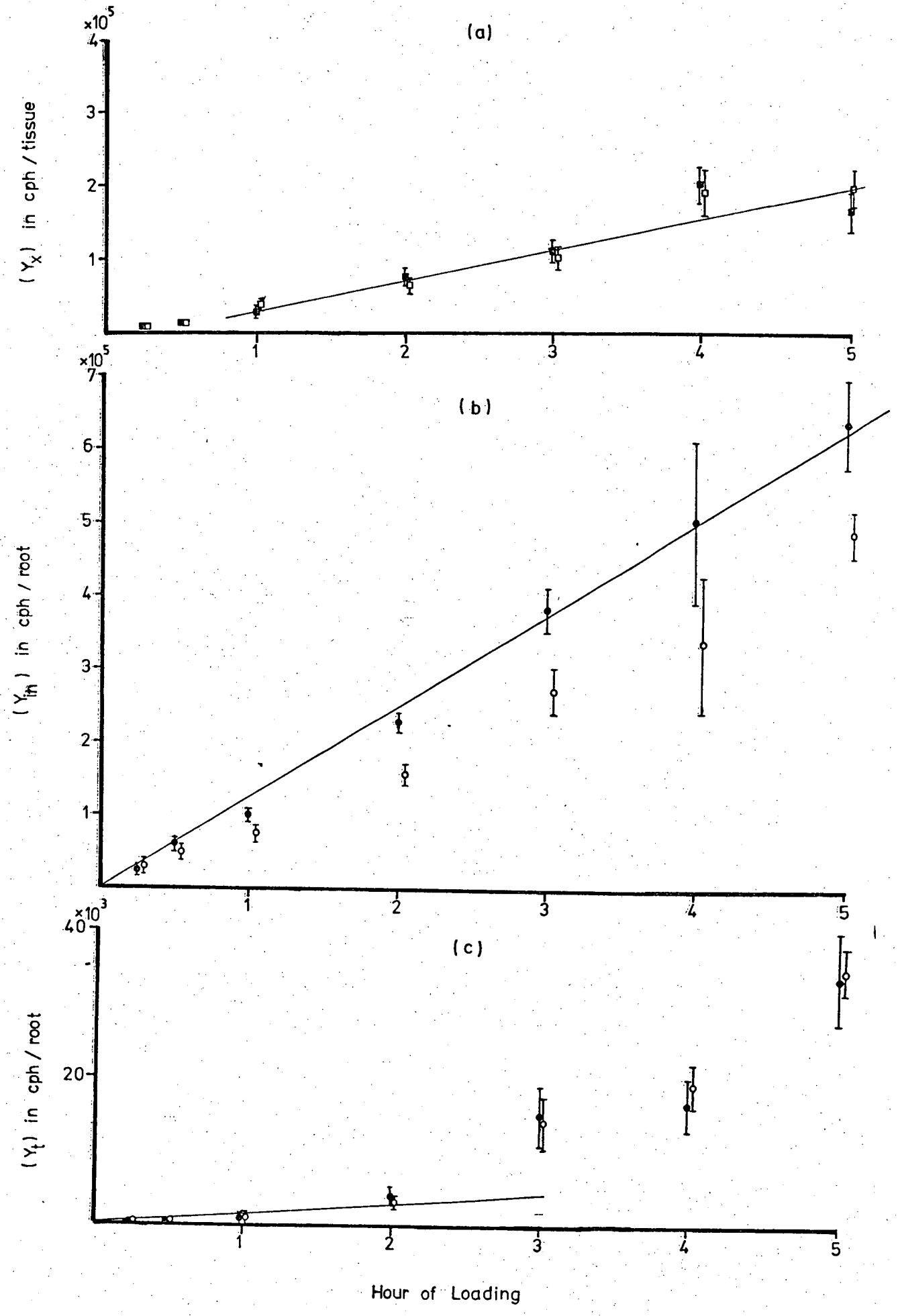
A comparison of the time course of ion uptake into a portion of the root, 10-20 mm from the tip, by using ^{86}Rb (open symbols) and ^{42}K (close symbols) isotopes. The limits are ± S.E. of the means from 5 observations. The average mass of the labelled tissue was 1.17×10^{-6} kg./root.

(a) transport into the shoot (Y_x)

(b) accumulation in the labelled root portion (Y_{in})

(c) transport to the non-labelled tip region (Y_t).

Note that the amount of tracer accumulated in the labelld tissue was analysed after 30 min wash in a K^+-free solution.


(a)
$\times 10^5$
4
3
2
1
(Y_x) in cph / tissue
1
2
3
4
5
(b)
$\times 10^5$
7
6
5
4
3
2
1
(Y_{in}) in cph / root
1
2
3
4
5
(c)
$\times 10^3$
40
20
(Y_t) in cph / root
1
2
3
4
5
Hour of Loading

build-up of specific activity of the tracer ions in the symplast.

A comparison of longitudinal transport toward the tip region (Y_t) between the two ion species is shown in Fig. 4.8(c). It appears that the amount found in the tip region for both tracers does not differ significantly over the 5 hr period. Initially, the rate of Y_t is fairly constant and increasing after 2 hrs of loading. If the initial rate is determined essentially by longitudinal symplastic transport, their similarity seems to confirm that there is no discrimination against Rb^+ at the plasmalemma. Moreover, it implies that ions transported to this region of roots are not from the vacuole; otherwise there would have appeared a greater Y_t for ^{42}K than ^{86}Rb in view of discrimination at the tonoplast.

At this stage, only the apparent influx (ϕ_{in}) can be estimated from the rate of tracer build-up in the labelled root tissue (Y_{in}) by taking into account the specific activity of the loading medium and the fresh mass of the root tissue. After combining the results from Fig. 4.7 and 4.8 for ^{86}Rb studies, values of dY_x/dt, ϕ_{in} and dY_t/dt for both tracers are shown in Table 4.2. The value of dY_x/dt and ϕ_{in} will be utilised in section 4.5.1 when ion fluxes and cytoplasmic content are estimated. The rate of transport into the xylem (ϕ_x) and to the tip region (ϕ_t) can be calculated (in sections 4.5.1 and 4.6.1, respectively) after the specific activity in the cytoplasm (s_c) is calculated.

4.4.4 The speed of ion transport in the xylem

A graph of tracer activities against time (Fig. 4.7) shows that the transport of ions into the xylem could be detected within 15 min. It is of interest to follow the short-term uptake along the xylem and to use it to determine the speed of movement up the xylem by using ^{86}Rb. The method of the measurements was described in section 4.3.4. After loading for 5 min or 10 min, the tissue above the labelled portion of the root was excised immediately into 2 mm segments and counted.

A typical graph of activities in these segments against the distance from the entry site is shown in Fig. 4.9, for 5 min and 10 min loading periods. Generally, it appears that

Table 4.2

Showing values of dY_x/dt and ϕ_{in} obtained from ^{86}Rb and ^{42}K uptake studies. These values are used to estimate fluxes in Table 4.7.

Note that ϕ_{in} was calculated from the ratio of dY_t/dt to s_o, after taking the root fresh weight into account.

	dY_x/dt	dY_t/dt	ϕ_{in}
	(cph.hr^{-1}/plant)		(m.equiv.kg^{-1}.hr^{-1})
^{86}Rb	.52x10^5	1.42x10^3	.80
^{42}K	.40x10^5	1.42x10^3	1.18

Table 4.3

Showing the distance (x), the speed of xylem flow (v) and the possible value of apoplastic transport (ϕ_{ox}) of intact roots when a portion of the root between 10-20 mm from the tip was labelled with ^{86}Rb, 40μCi/ml activity, for 5 minutes and 10 minutes.

T = loading time and s_o = 1.5x10^{12} cpm.mole^{-1}

* = data shown in Fig. 4.9

T min.	x mm.	v mm.min^{-1}	Y_{ox} cpm.min^{-1}	ϕ_{ox} moles.min^{-1}.mm^{-1}
5 *	7.8	1.56	.9	.12x10^{-12}
	7.8	1.56	.6	.08x10^{-12}
	9.8	1.96	.9	.12x10^{-12}
	9.8	1.96	1.0	.13x10^{-12}
$\bar{X}$ ± S.E.	8.80±.58	1.76±.12	.83±.08	(.11±.01)x10^{-12}
10	15.8	1.58	1.6	.11x10^{-12}
	17.0	1.70	1.0	.07x10^{-12}
	17.0	1.70	1.6	.11x10^{-12}
*	15.0	1.50	1.1	.07x10^{-12}
$\bar{X}$ ± S.E.	16.20±.49	1.62±.05	1.33±.16	(.09±.01)x10^{-12}

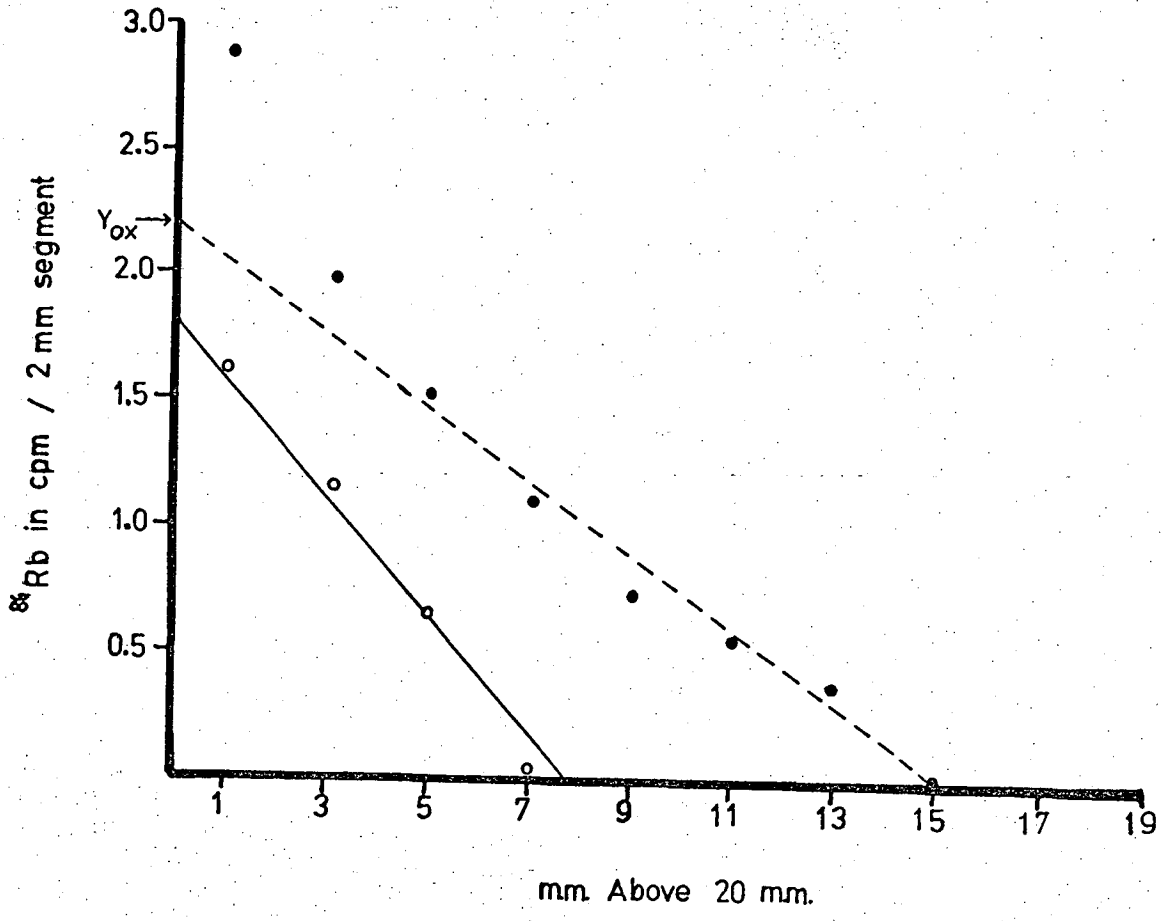
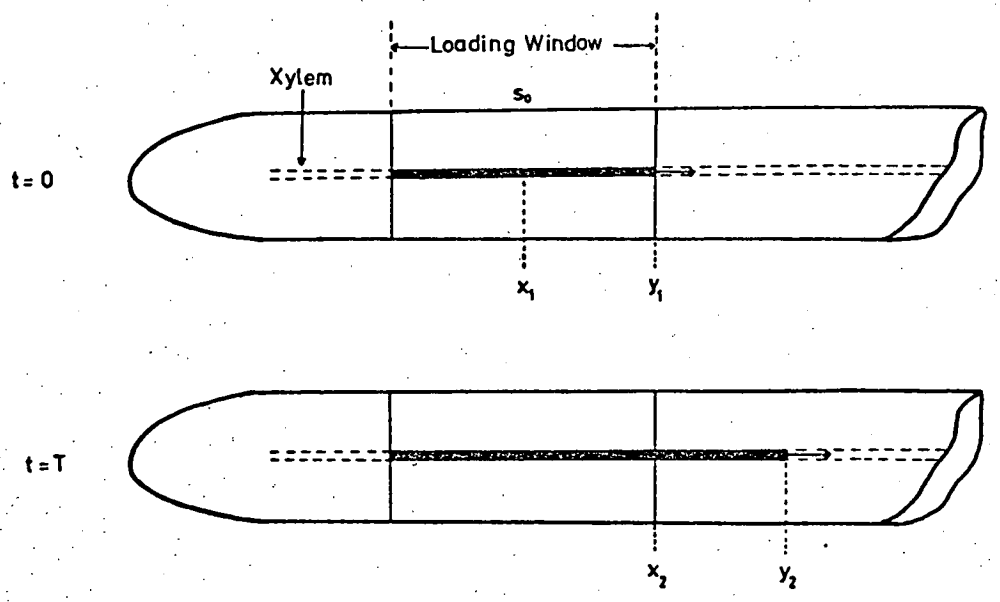


Fig. 4.9.

A typical graph of ion distribution along the root tissue above the labelled region (10-20 mm) of the intact root, after 5 (o) and 10 (●) min loading. Data represents ^{86}Rb activity found for each 2 mm of a single root. Y_{ox} is the amount emerging from the labelled portion at the end of the loading. The standard s_o was 1.5×10^{12} cph.mole^{-1} of K^+.

segments closer to the labelled portion contain greater activities. There is a distinct front beyond which the tracer has not travelled and this is about twice as far from the loading region for 10 min than for 5 min. The distance and the speed of ion movement in the xylem (v_x) are shown in Table 4.3. From 8 observations, the average speed of transport is $1.7 \pm .06$ mm.min^{-1}.($\pm$S.E.). The fact that the front has moved twice as far up the root in 10 min than in 5 min indicates that some tracer is moving rapidly across the root to the xylem. The movement is likely to be either via the apoplast or transported by vesicles in the symplast (Pitman, personal communication), since this period is too short to account for symplastic transport. Note that the half time ($.693/k_s$) for equilibration with the symplast is 50 min. Hence, the slope of the graph is $s_o\phi_{ox}$.

It should be noted that apoplastic transport into the xylem of rice roots is possible in the region studied, since complete suberization of the endodermis was found at distances of the about 40-50 mm from the tip (see Kawata and Lai 1968, and Yoshida 1981). If this is the case, the magnitude of apoplastic transport (ϕ_{ox}) can be determined as follows:



Consider a column of the xylem sap, from the above diagram, which at the start of loading is at the position X_1, Y_1. At the end of loading, the column moves to X_2, Y_2. The activity in the segment at X_2 has arisen due to the exposure to a direct flux from the medium, $s_o\phi_{ox}$. For the whole period of time T,

$$Y_{ox} = s_o\phi_{ox}\, T \qquad (4\text{-}1)$$

where Y_{ox} is the activity per unit root length of the root and, hence, ϕ_{ox} is the apoplastic flux per unit length. The calculated values of ϕ_{ox} are also shown in Table 4.3. On average, ϕ_{ox} is 0.10×10^{-12} moles.min^{-1}.mm^{-1}. This is 0.05 m.equiv.kg^{-1}.hr^{-1} when the fresh mass of the labelled tissue is taken into account.

4.4.5 Tracer studies of K^+ accumulation in non-labelled portions of roots : A comparison between the efflux from the xylem (Y_{xc}) and longitudinal transport.

This section describes a method of distiguishing longitudinal transport between cortical cells from xylem efflux in a segment of root above the labelled tissue. The measurements of these two were made in the same plants and it was assumed that longitudinal transport toward the basal part of the root was equal to that toward the tip region. If longitudinal transport to the non-labelled tissue (section 4.4.3) was caused mainly by the transport in the apoplast, root excision at 2 mm away from the labelling site may not be far enough to eliminate this effect. The following experiment was designed (see section 4.3.5) in such a way that tracer activities found in 10 mm root segments between 0-10 mm and 30-40 mm from the tip were to be compared at the end of each loading period and the root was labelled between 15-25 mm from the tip. For convenience, activities in 0-10 mm and 30-40 mm segments would be designated as Y_{down} and Y_{up}, respectively.

The results are shown in Fig. 4.10. As is shown, Y_{up} increases at a fairly steady rate over the 5 hr period. Although Y_{down} is smaller at the beginning, it is observed at an increasing rate after the first hour and becomes greater than Y_{up} after 4 hrs. If this increasing rate is due to the re-translocation from the shoot via the phloem, its earlier appearance compared to that from the previous study (i.e. 3 hrs in section 4.4.3) is partly due to the shorter distance of the labelled tissue from the seed than in the previous case. Since the increase in Y_{up} is not of the same magnitude, it can be concluded that phloem transport has a greater effect on accumulation in the young cells of the tip region than in the mature cells.

The best estimate for the rate of accumulation of tracer in the tissue outside the loading region is from the linear part of Y_{down} (i.e. at the end of 1 hr loading). On a per 10 mm length basis, Y_{down} is 3.8×10^3 cph.hr^{-1}, while the corresponding rate of Y_{up} over the same period of time is 9.6×10^3 cph.hr^{-1}. The difference between these two is, therefore,

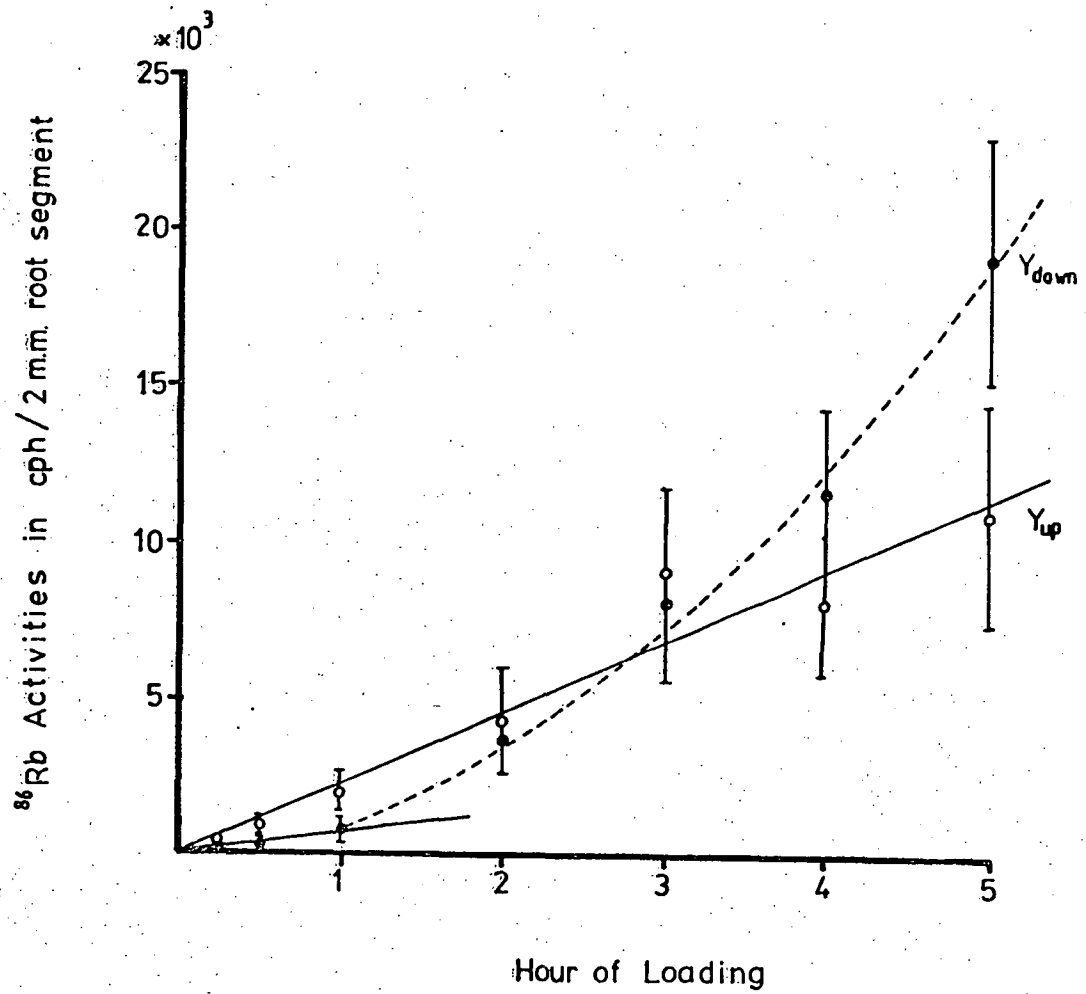


Fig. 4.10.

Time course of ^{86}Rb accumulation in a 2 mm root segment after being washed in a non-labelled solution for 1 hr. Y_{down} is the accumulation in a segment below the labelled portion of the root, and Y_{up} is that in a segment above the portion.

The limits are ± S.E. of the means from 5 observations. $s_o = 0.9 \times 10^{14}$ cph.mole^{-1}.

the outflow from the xylem (Y_{xc}) which is 5.8×10^{3} cph.hr^{-1}. This information by itself is of little value. To determine ϕ_{xc} (the efflux from the xylem to the cytoplasm), it is necessary to know the specific activity of the xylem sap (section 4.4.7).

4.4.6 Estimates of the rate of ion transport in the xylem (dQ_x/dt)

An attempt was made to estimate the xylem flux from the rate of increase in K^+ content of the upper part of the plant (together with any loss from the tissue to the bathing medium). This was done by exposing part of the root between 0-20 mm from the tip to a 1x solution, while the rest of the root was in a K^+-free solution. After being set up horizontally, plants were allowed to adjust to the environment for 5 hrs. Solutions in all chambers were, then, changed to fresh ones, and the experiment was started from this moment. One group of plants was harvested at 0 hr and another at 50 hrs later. Fresh solutions were provided every 12 hrs to avoid undue change in concentration and possible re-absorption into the basal part of the root. The solution bathing the upper part of the plant was analysed for any build-up which must have come from the tissue. K^+ content in the treated tissue (i.e. initially between 0-20 mm from the tip) was also recorded for comparison.

Table 4.4 shows the average K^+ content in the treated tissues (Q_r) and in the tissue above the initial 20 mm from the tip (Q_x) from 4 observations. The result shows that Q_x increases at a greater rate than Q_r. Since the source of K^+ ions for growth is the solution bathing the plant or from the existing 0-20 mm root tissue, the result suggests that after the ions are absorbed into the root most of them are transported to the shoot. The rate of K^+ transport can be determined from dQ_x/dt which is shown to be 23.8×10^{-9} moles.hr^{-1}. It was noted that the initial 0-20 mm length of the root (at 0 hour) grew on average to about 40.5 mm at the end of 50 hrs. The increased length of the root at the latter period of the experiment naturally includes a proportionately greater development of xylem thus leading to considerable overestimation of dQ_x/dt from the average value

during the 50 hrs. In section 4.4.3 and 4.4.5, the possibility of downward transport via the phloem was raised. It can be seen from Table 4.4 that if such transport occurs it can, at most, be only a small fraction of the upward xylem transport for this plant age. A further attempt was made by measuring dQ_x/dt over shorter periods of time (i.e. 14 hrs and 24 hrs). It was found that no reliable estimates of dQ_x/dt could be made.

Another approach to obtain dQ_x/dt is from a knowledge of ion concentration in the xylem (C_x). Its relation to dQ_x/dt is

$$dQ_x/dt = C_x \cdot A_x \cdot v_x \qquad (4\text{-}2)$$

where A_x is the xylem cross section area, and v_x the speed of flow in the xylem.

To measure C_x, an attempt was made by collecting the exudate from freshly cut ends of roots (Davis and Higinbotham 1976) or shoots (Pitman 1965 a) using a 2 microliter capillary tube. The roots were bathed vertically in distilled water under a saturated atmospheric chamber. Over a 5 hr period, it was not possible to detect any exudate by capillary action. This could be a consequence of slow growing roots (Jefferies 1973) and, therefore, a relatively small water uptake. Note that the rate of root growth for barley was 1.2 $mm.hr^{-1}$ (Weisenseel et al. 1979), about twice of that for rice. Due to this problem, the collection was made from the guttation of the shoot apex instead. This was based on the assumption that ion concentration in the xylem was uniform over the whole length of the root, and a droplet appears at the shoot apex is the exudate from the xylem. The possibility of an overestimation of the xylem concentration obtained by this method is discussed below. After 4 days of growing, the growing system of the plants was covered overnight to reduce the humidity of the surrounding atmosphere. On the next morning, droplets from 15 seedlings were used to fill the 2 microcapillary tube. The solution was diluted to a known volume and K^+ content was analysed by Atomic Absorption Spectrophotometry. The average concentration obtained by this method was 42.5 mM/root.

Table 4.4

A comparison of K^+ content in seedlings at 0 hour and 50 hours after the commencement of the experiment. Q_r: that of the root tissue (0-20 mm from the tip) which was exposed to a 1x solution, Q_x: that of the upper part of the plant (i.e. beyond 20 mm from the root tip) which was partly exposed to a K^+-free solution (the basal part of the root) and partly to air (the shoot).

The limit denotes standard error of the means from 4 observations, 16 plants in total. T is time after commencement of experiment.

T (hr)	Q_r	Q_x	Total
	(moles x 10^{-6} / tissue)		
0	.55±.07	9.70±.28	10.25±.27
50	.84±.18	10.89±.31	11.74±.38
Rate in (moles.hr^{-1})	5.8×10^{-9}	23.8×10^{-9}	29.8×10^{-9}

Table 4.5

Comparing K^+ content between plants grown for 44 hrs in a K^+-free solution [B] and in a 1x solution [C] with that of 0 hour plants [A] (i.e. at the commencement of experiment). Each sample was taken from 5 plants.

	T (hr)	whole root	seed	shoot	Total
		(moles x 10^{-6} / plant)			
[A]	0	.86	1.31	1.94	4.11
[B]	44	1.46	.90	2.35	4.71
[C]	44	2.43	1.20	3.43	7.06

Information on the cross sectional area of the xylem vessels was obtained from transverse sections of the root between 10-20 mm from the tip. This area was typically 2.55×10^{-9} $(m)^2$, about 1.3% of the total root cross section area (chapter 3). The speed of ion transport in the xylem obtained from section 4.4.5 was 0.10 $m.hr^{-1}$. Based on these values, the calculated value of dQ_x/dt is 10.85×10^{-9} $moles.hr^{-1}$.

The xylem concentration obtained by the guttation method, however, could be an overestimate due to the possibility of some evaporation of the droplets before collection and to the possible supply of ions from the seed.

To test the dependence of 5 day old plants on ion supply from the seeds, plants at that age were separated into two groups; one was grown in a K^+-free solution, and the other in a 1x solution for a comparison. K^+ content in both groups of plants was compared with that at 0 hrs. Averaged from 5 seedlings and from a single observation over a 44 hr period, the result showed that the reduction in K^+ content of the seed of plants grown in the K^+-free solution was about 22% more than that of plants grown in the K^+ containing solution (see Table 4.5). That of the former was concomitant with the increase in the shoot and root content, confirming the overestimation of C_x obtained by the guttation method. The most appropriate value of C_x is suggested in section 4.4.8. This result also indicates the existence of phloem transport to the roots of 5 day old seedlings.

4.4.7 The specific activity in the xylem (s_x)

In the previous section, two estimates have been made of the rate of xylem transport. These are 23.85×10^9 moles.hr^{-1} by direct estimation of tracer build-up in the upper part of the plant and 10.80×10^9 moles.hr^{-1} by the guttation method. Despite the arguments which suggest that both of these values are overestimated, they are now used to estimate the specific activity in the xylem (s_x).

As shown in chapter 2, the value of s_x is determined by

$$s_x = \frac{dY_x/dt}{dQ_x/dt} \qquad (2\text{-}36)$$

From the studies using ^{86}Rb, dY_x/dt was determined to be 0.75×10^5 cph.hr^{-1} (Fig. 4.7). Under the study conditions, the tip region of the root was exposed to a non-labelled solution. This could cause some dilution to the activity in the xylem, due to uptake of water and non-labelled ions.

Utilising the dQ_x/dt values, the corresponding s_x values are $0.03s_o$ and $0.08s_o$, respectively. These values are much less than the estimated value of s_c (= $0.30s_o$) reported in section 4.5.1. Although these values of s_x are underestimated, a most probable value which is suggested in section 4.4.8 (i.e. $.13s_o$) is also small compared to s_c value. This result is in contrast to the estimate for low salt roots in which $s_x = s_c = s_o$ (Pitman 1971, Behl and Jeschke 1982 and Jeschke 1982).

4.4.8 Estimates of the values of ion efflux from the xylem (ϕ_{xc})

Work described in section 4.4.5 shows that it is possible to measure tracer efflux from the xylem. The rate of tracer ions increasing in the tissue due to the efflux is 0.57×10^4 cph.hr^{-1} per 10 mm root length. When this rate was compared to that of tracer transport into the shoot (Fig. 4.7),

Table 4.6

Showing possible values of ϕ_{xc} for different dQ_x/dt and s_x/s_o values, using $dY_x/dt = .75 \times 10^{5}$ cph.hr^{-1} (Fig. 4.7) and $dY_{xc}/dt = .57 \times 10^{4}$ cph.hr^{-1} (section 4.4.5).

dQ_x/dt was calculated from $C_x \cdot A_x \cdot v_x$, per root basis (see text - section 4.4.6). $s_o = .90 \times 10^{14}$ cph.mole^{-1} K^+ and the average mass of a 10 mm root segment was 1.22×10^{-6} kg.

	C_x (mM)	dQ_x/dt (moles.hr^{-1})	s_x/s_o	ϕ_{xc} (m.equiv.kg^{-1}.hr^{-1})	Note
(a)	93.3	23.80×10^{-9}	.03	1.73	chemical assay
(b)	42.5	10.85×10^{-9}	.08	.65	guttation method
(c)	25.0	6.37×10^{-9}	.13	.40	average of b and d
(d)	7.6	1.94×10^{-9}	.43	.12	if $s_x = s_c$

it was found to be about 7 times smaller during the lag period, and about 13 times smaller at quasi-steady state. However, it would be wrong to conclude that ϕ_{xc} is much smaller than ϕ_{cx}, since the previous section showed that $s_x << s_c$.

Taking the specific activity of the xylem into account, ϕ_{xc} can be estimated from

$$\phi_{xc} = (dY_{xc}/dt)/s_x \qquad (4\text{-}3)$$

Table 4.6 shows that ϕ_{xc} is sensitive to the change of dQ_x/dt. Due to a possible overestimation of dQ_x/dt, the value obtained from $s_x = 0.03s_o$ and $s_x = 0.08s_o$ are, thus, overestimated. The finding of some K^+ contribution from the seeds to 5 day old shoots in section 4.4.6 (see Table 4.5) suggests that the specific activity in the xylem should not be much less than that of the symplast. If $s_x = s_c$, the corresponding value of ϕ_{xc} is 0.12 m.equiv.kg^{-1}.hr^{-1}, which is underestimated. Generally. the smaller value for s_x than for s_c is expected in high salt roots due to the efflux of ions from the xylem to the symplast. Evidence for this can be seen from Davis and Higinbotham (1976). It is concluded that the possible value of ϕ_{xc} is between 0.12 m.equiv.kg^{-1}.hr^{-1} and 0.65 m.equiv.kg^{-1}.hr^{-1}. Taking the mean of these values, it is 0.40 m.equiv.kg^{-1}.hr^{-1}. Apparently, this value corresponds with xylem concentration of 25 mM, the same as that of barley roots (Pitman 1965a) and with s_x of $0.13s_o$. Also note that xylem concentration in corn roots, reported by Davis and Higinbotham (1969), was 23 mM.

4.5 Data Analysis

4.5.1 Estimation of ion fluxes and cytoplasmic content

Because it was observed that there was a net ion loss from the studied portion of the root grown horizontally (i.e. ϕ_v = -0.79 m.equiv.kg^{-1}.hr^{-1}, from Fig. 4.7), the following estimates of fluxes and the internal ion contents utilised the analytical procedure for non-steady state conditions (chapter 2, section 2.2.2). The equations used are as follows:

$$\phi_{co} = \left[\left(\frac{\gamma}{2\alpha'} \right)^2 + \frac{\phi_{cx}\phi_{in}}{\alpha'} \right]^{1/2} - \frac{\gamma}{2\alpha'} \qquad (2\text{-}33)$$

$$\phi_{cv} = \alpha' \phi_{co} \qquad (2\text{-}31)$$

$$Q_c = \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{k_s} \qquad (2\text{-}19)$$

$$s_c \simeq \frac{s_o \phi_{oc}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}34)$$

where $\phi_{oc} = \phi_{co} + \phi_v + (\phi_{cx} - \phi_{xc})$

$\alpha' = s_o \phi_{in} / k_s Y_s'$

$\gamma = (\phi_v + \phi_{cx} - \phi_{xc})\alpha' - (1 + \alpha')\phi_{in}$

and $\phi_v = (\phi_{cv} - \phi_{vc})$

Further information required for use in the above equations is the value of ϕ_{cx}, the rate of ion transport into the xylem. This can be obtained from the rate of tracer build-up in the xylem which is given by

$$dY_x/dt = (s_c\phi_{cx} - s_x\phi_{xc}) + s_o\phi_{ox} \qquad (4\text{-}4)$$

It has been argued that $s_x\phi_{xc}$ (section 4.4.7 and 4.4.8) and $s_o\phi_{ox}$ (section 4.4.4) are each small in comparison to $s_c\phi_{cx}$. Hence, ϕ_{cx} can be calculated from

$$\phi_{cx} \simeq (dY_x/dt)/s_c \qquad (4\text{-}5)$$

At this stage the value of s_c is not known and ϕ_{cx} cannot be calculated from the value of dY_x/dt. The method of proceeding is to choose a plausible value of s_c and use this to calculate ϕ_{cx}. The ϕ_{cx} value is then used in the calculations for values of the unknown fluxes and a value of s_c. If the calculated

Table 4.7

Estimations of fluxes (ϕ) and the cytoplasmic content (Q_c) of K^+ ions using (a) ^{86}Rb and (b) ^{42}K as a tracer when ϕ_v = -.79 m.equiv.kg^{-1}.hr^{-1}, ϕ_{xc} = .40 m.equiv.kg^{-1}.hr^{-1} utilising k_e and Y_e' from Table 4.1. The value of dY_x/dt and ϕ_{in} used in these calculations were from Table 4.2.

T = tissue loading time in hr, ϕ in m.equiv.kg^{-1}.hr^{-1} and Q_c in m.equiv.kg^{-1}

(a) ^{86}Rb washout

	Exp.	T	ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}	Q_c	s_c/s_o	ϕ_{cx}	β
	1	2	1.62	1.18	2.73	3.52	8.80	.29	1.63	2.38
	2		1.80	1.51	2.40	3.19	7.18	.32	1.48	1.98
*	3	5	3.61	3.98	1.37	2.16	5.14	.58	.82	1.21
	4		2.53	2.71	1.71	2.51	10.1	.47	1.01	1.37
	5		3.14	3.45	1.48	2.27	5.99	.54	.88	1.26
	6		1.38	.51	3.56	4.35	8.63	.23	2.06	5.07
	7		2.07	2.01	2.05	2.84	7.70	.38	1.25	1.62
	8		1.68	1.29	2.61	3.40	6.52	.30	1.58	2.23
	9		2.04	1.98	2.09	2.88	5.26	.38	1.25	1.63
	10		1.42	.55	3.38	4.17	7.88	.23	2.06	4.77
	11	24	4.06	4.49	1.29	2.08	10.9	.62	.76	1.17
	12		10.9	11.5	.96	1.75	10.7	.84	.56	1.05
	$\bar{X}$**		2.13	1.92	2.34	3.13	7.32	.37	1.40	2.35
	±		±	±	±	±	±	±	±	±
	S.E.		.24	.37	.23	.23	.51	.04	.14	.45

(b) ^{42}K washout

Exp.	T	ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}	Q_c	s_c/s_o	ϕ_{cx}	β
1	5	1.98	1.65	4.68	5.47	9.23	.25	1.52	1.92
2		2.35	2.39	3.56	4.35	10.8	.33	1.15	1.48
3		1.97	1.70	4.72	5.51	10.9	.26	1.46	1.86
4		2.29	2.29	3.70	4.49	7.48	.32	1.19	1.52
5		2.30	2.30	3.68	4.47	7.39	.32	1.19	1.52
$\bar{X}$		2.18	2.07	4.07	4.86	9.16	.30	1.30	1.66
±		±	±	±	±	±	±	±	±
S.E.		.08	.16	.26	.26	.76	.02	.08	.09

Note

* is data shown in Fig. 4.6

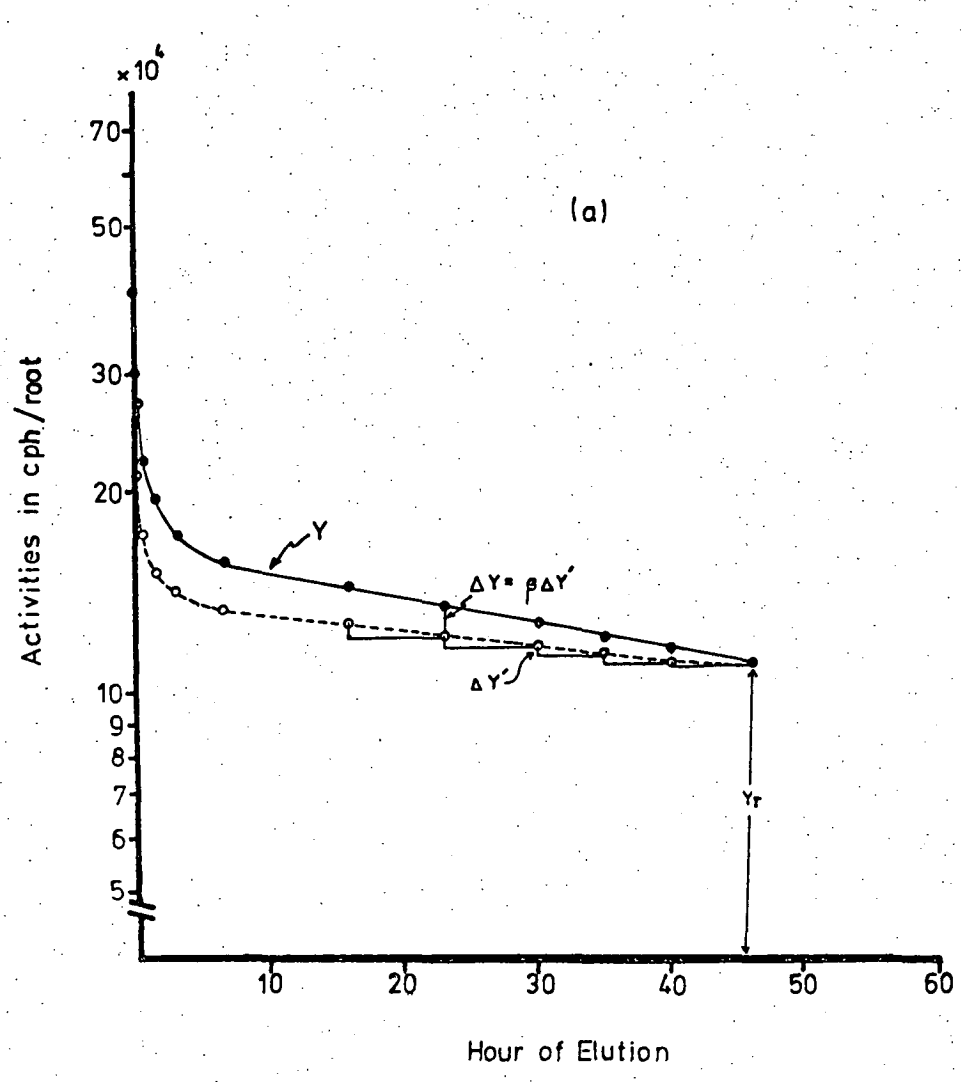
** = not include the 24 hrs loading

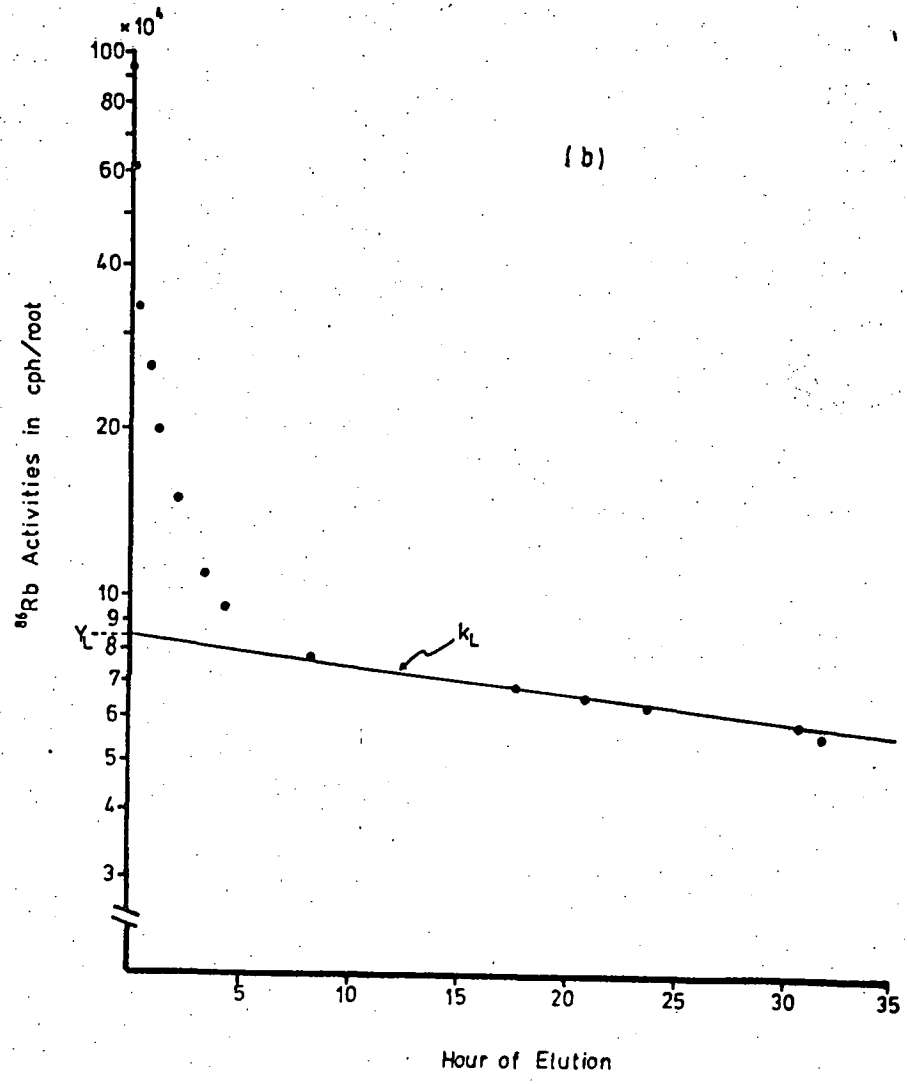
$\beta = (\phi_{co} + \phi_{cx}) / \phi_{co}$ (was used in section 4.5.2)

Fig. 4.11.

(a) Showing a construction of tracer remaining in a labelled portion of the root. The tracer lost to the bathing medium in each elution sample ($\Delta Y'$) is scaled up by the β factor and these values are progressively added to the residual amount left in the tissue at the end of the washout experiment (Y_L) so that the graph of Y against time can be constructed.

(b) Time course of tracer remaining in the washed tissue, after correction for the fraction of transport into the shoot (β). The slope is the rate of long-term exchange (k_L), and Y_L is the total amount of tracer appearing in the vacuole at the end of loading. Data shown was from experiment #3 in Table 4.1.


(a)
×10⁴
70
50
30
20
10
9
8
7
6
5
Activities in cph/root
Y
ΔY = βΔY'
ΔY'
Y_T
10
20
30
40
50
60
Hour of Elution


(b)
×10⁴
100
80
60
40
20
10
9
8
7
6
5
4
3
⁸⁶Rb Activities in cph/root
Y_L
k_L
5
10
15
20
25
30
35
Hour of Elution

value of s_c differs from the value used previously, a correction is made and the calculations are repeated until agreement is reached. These iterations are completed in less than a minute, using a programmable calculator (HP-97).

The following analysis was made utilising data of k_s and Y_s' from Table 4.1 and dY_x/dt and ϕ_{in} from Table 4.2. The estimated fluxes, the cytoplasmic content, the corresponding s_c for ϕ_{cx} and a fraction of transport into shoots ($\beta = \phi_{co}+\phi_{cx}/\phi_{co}$) are shown in Table 4.7(a) and (b) for $K^+(^{86}Rb)$ and $K^+(^{42}K)$, respectively. The observations on which these calculations are based are also given in the table. For a longer period of loading, s_c tends to be closer to that of the loading medium (s_o). However, the values obtained from 24 hr loading experiments were not used in the averages because the values of dY_x/dt and ϕ_{in} were determined under conditions more appropriate to the short-term loading experiments.

Note that in all of the individual experiments tabulated in Table 4.7, there is a net loss from the vacuole (i.e. $\phi_{vc} > \phi_{cv}$) and there is a net transport via the xylem (i.e. $\phi_{cx} > \phi_{xc}$). If the latter net flux is greater than the former, there is a net uptake from the bathing medium (i.e $\phi_{co} > \phi_{oc}$), whereas in a few cases the reverse is true and, hence, $\phi_{co} > \phi_{oc}$.

4.5.2 The rate of long-term exchange and the total tissue content

From chapter 2, the total tissue content is given by

$$Q = \frac{\phi_{vc}}{k_L}\left(1 - \frac{\phi_{in}}{\phi_{oc}}\right) + \frac{\phi_{oc} + \phi_{xc}}{k_s} \qquad (2\text{-}21)$$

The value of Q can be estimated providing that the amount of tracer in the tissue in a long-term washout study can be determined. This can be obtained if a graph of the amount of tracer remaining in the washed tissue with time can be plotted

(on a semi-logarithmic scale). To construct this graph, the tracer lost to the bathing medium in each elution sample ($\Delta Y'$) is scaled up by the β factor. These values are progressively added to the residual amount left in the washed tissue (Y_r) at the end of each washout experiment so that the graph of Y against time can be constructed. The graph is shown in Fig. 4.11. It is usually linear over the range of 5-20 hrs of washing, depending on the sample. The slope obtained from the linear part of the graph represents the rate of long-term exchange (k_L). Extrapolation to t=0 gives the total amount of tracer in the long-term component (Y_L).

It is worth noting that some workers have observed that the washout graph is not a simple summation of exponential components but has irregularity form during the early stage of transient components. The cause of these discontinuities has been considered by Pallaghy et al. (1970), Erlandson (1979) and Jensen and Kylin (1980).

Values of k_L and the estimated Q and Q_v (= $Q-Q_c$) are shown in Table 4.8(a) and (b) for $K^+(^{86}Rb)$ and $K^+(^{42}K)$, respectively. In cases when k_L of $K^+(^{42}K)$ and $K^+(^{86}Rb)$ are obtained from the same tissue, that of the latter is always greater. In equation (2-21), the second term which involves k_e is always small in comparison with the first term in which it is seen that Q is inversely proportional to k_L. On average, Q_v of $K^+(^{86}Rb)$ is 123 m.equiv.kg^{-1}, while that of $K^+(^{42}K)$ is 231 m.equiv.kg^{-1}, about 1.8 times greater.

The suitability of ^{86}Rb as a tracer for K^+ ions can be tested by comparing the total content Q estimated in the tracer studies with the value obtained from chemical assay. The latter was averaged over 0-24 hrs (Fig. 4.5a) is 240 ± 15 m.equiv.kg^{-1}. This value is far greater than the average value of $K^+(^{86}Rb)$. This is due mainly to the smaller in the rate of the apparent influx (ϕ_{in}) and the greater long-term exchange rate (k_L). The total tissue content (Q) of K^+ obtained from ^{42}K tracer studies agrees well with the assayed value.

Table 4.8

Showing values of k_L, Q and Q_v obtained from (a) ^{86}Rb and (b) ^{42}K washout, utilising values from Table 4.7.

(a) ^{86}Rb washout

Exp. no.	T (hr)	k_L (hr^{-1}) $x10^{-2}$	Q (m.equiv.kg^{-1})	Q_v
1	2	1.06	171.3	162.5
2		1.91	95.7	88.5
3	5	1.18	145.8	140.7
4		1.13	157.3	147.2
5		1.55	112.8	106.8
6		1.07	173.4	164.8
7		1.51	119.0	111.3
8		1.41	128.8	122.3
9		1.89	95.0	89.7
10		1.83	101.9	94.0
$\bar{X}$ ± S.E.		1.45 ± .11	130.1 ± 9.5	122.8 ± 9.3

(b) ^{42}K washout

Exp. no.	T (hr)	k_L (hr^{-1}) $x10^{-2}$	Q (m.equiv.kg^{-1})	Q_v
1	5	.86	256.8	247.6
2		.96	229.8	219.0
3		.95	235.9	225.0
4		.98	224.9	217.4
5		.87	253.0	2456.6
$\bar{X}$ ± S.E.		.93 ± .02	240.1 ± 6.3	230.9 ± 6.5

Fig. 4.12 compares ion fluxes and the internal contents obtained from ^{42}K and ^{86}Rb studies. It shows that fluxes across the plasmalemma for $K^+(^{42}K)$ are slightly greater than those for $K^+(^{86}Rb)$, but not significantly so. Although the cytoplasmic specific activity (s_c) for $K^+(^{42}K)$ is smaller due to the greater exchange of the ion species across the tonoplast, the xylem inward flux does not seem to be affected. The greater fluxes to and from the vacuole for $K^+(^{42}K)$ than $K^+(^{86}Rb)$ are in agreement with the greater estimated vacuolar content in the former case. This result clearly indicates that the tonoplast is the major site to discriminate Rb^+ from K^+ ions.

It is interesting to note that at the end of a 5 hr loading period, the specific activity of the cytoplasm is only 30%-37% of that of the loading medium, whereas it is much less in the xylem (i.e. 0.08%). Consequently, the method used by Pitman (1971), Behl and Jeschke (1982) and Jeschke (1982), where it was assumed that $s_x = s_c$, are not valid in these experiments in which the roots are not in the low salt conditions.

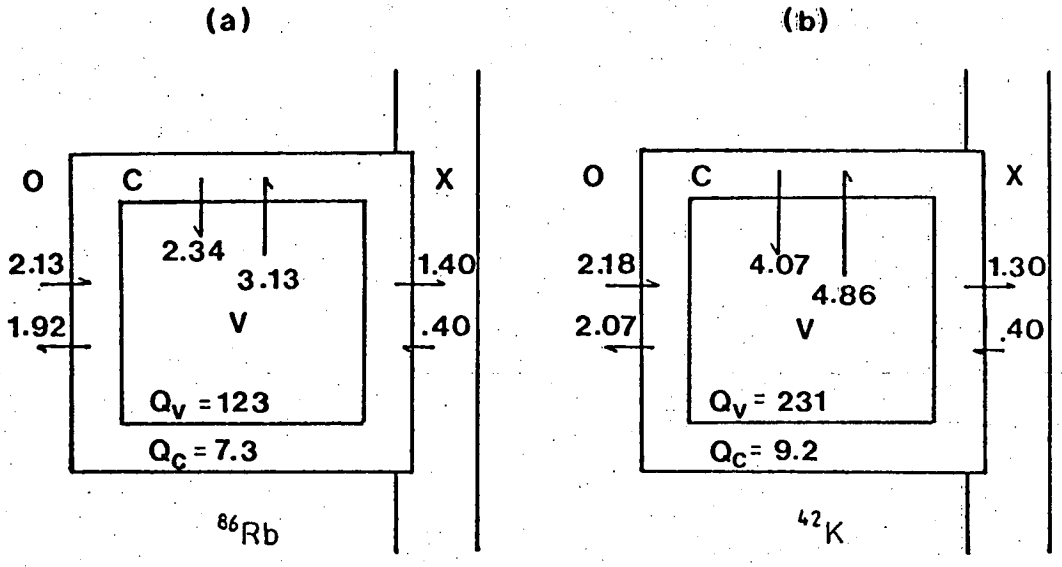


Fig. 4.12

A comparison of fluxes (half arrows) across the plasmalemma (ϕ_{oc} and ϕ_{co}), the tonoplast (ϕ_{cv} and ϕ_{vc}) and the xylem (ϕ_{cx} and ϕ_{xc}) and the internal contents (Q_c and Q_v) of K^+ ions in the mature portion of an intact root.

(a) when ^{86}Rb was used as a tracer for K^+ ions

(b) when ^{42}K was used.

O represents the external medium, C; the cytoplasm, V; the vacuole, and X; the xylem vessels.

Flux meqiv kg^{-1} hr^{-1}
Q meqiv kg^{-1}

4.6 Discussion

This study has shown that a simple mathematical treatment based on the Pitman model can be used to estimate ion fluxes and concentrations in a mature part of the root. It is not necessary that the roots under study be under steady state conditions and the methods allow for a net change in vacuolar content during the experiment. The existence of an alteration from steady state conditions (i.e. $\phi_v \neq 0$), after plants were arranged horizontally, provides a good opportunity to test mathematical analysis for non-steady state conditions of root tissue.

The analysis shows that there is no net uptake into this region of the root. This is rather surprising since the root serves as an absorption organ for the plant. It has been suggested that besides absorption via the epidermis, direct absorption of ions from the apoplast into the cortical cells was possible (Pitman 1977). Since the collapse of cortical cells which is associated with the formation of large air spaces (chapter 3, section 3.4.2) has not yet occurred in this region of the root, the small absorption suggests that cortical cells may lose their absorption ability, probably due to cell differentiation (Van Iren and Boers-Van der Sluijs 1980b). As a consequence, most of the ions transported into the shoot are derived from the reserve in cell vacuoles. This conclusion is supported by the finding of a gradual loss of K^+ from the tissue (Fig. 4.5). The root cells, therefore, act as a reserve of K^+ from which the developing shoot may draw at this stage of plant growth. Alternatively, the loss of the tissue content which is concomitant with the greater shoot growth rate (section 4.4.1) could be a characteristic of horizontally grown plants, since this was not found in vertically grown ones-at least during the experimentation period. It is possible that the orientation of plants or the water repellant grease may have caused the net ion loss from this region of the root and enhanced the shoot growth. In addition, as the solution was replaced with a fresh one every 12 hrs, the loss of K^+ from the tissue is not likely to be due to

an increase in the pH value of the medium (Zsoldos and Erdei 1981).

Fig. 4.8(b) shows that the accumulation of tracer in the tissue is near-linear over a 5 hour period provided that the root has been washed for 30 minutes to remove tracer in the free space. The slope of this graph was used to give ϕ_{in}. By using the equations in section 4.5.1, it was found that the plasmalemma fluxes and the compartmental contents were insensitive to the value of ϕ_{in}, but its value did affect the tonoplast fluxes. For example, a 10% change in ϕ_{in} caused about 15% change in the unidirectional tonoplast fluxes.

The main weakness in the data that has been used in this study is the estimation of ϕ_v and ϕ_{xc}. Both determinations require the measurements of K^+ content of plant tissue and this can only be done in studies of different plants of various ages. Variability between plants limits the accuracy with which these changes can be estimated. Since the range of the possible value of ϕ_{xc} is rather large (i.e. from .12 m.equiv.$kg^{-1}.hr^{-1}$ to .65 m.equiv.$kg.^{-1}.hr^{-1}$), a more satisfactory method to estimate the xylem concentration is possibly by using a K^+ sensitive electrode (Thomas 1978 and references therein). However, problems may arise, due to a tough wall of endodermal cells, if intact roots are used (see chapter 6). Despite these difficulties, the agreement found between the K^+ content of the tissue made by direct chemical assay (section 4.4.1) and by the tracer methods (when ^{42}K but not ^{86}Rb, is used as a tracer) gives confidence in both the methods and in the data used in the analysis.

It is of importance to note that $s_x << s_c$ at all stages of the investigation and, hence, $s_x\phi_{xc}$ is never more than about 10% of $s_c\phi_{cx}$. The effect of including this term causes changes in the estimates of the fluxes which are even smaller than 10%. It is, therefore, justified to neglect $s_x\phi_{xc}$ in the equation (4-4). However, if ϕ_{xc} was assumed in the present study to be negligible as done by many authors (Pitman 1971, Davis and Hinginbotham 1976, Jeschke 1977, Jeschke and Stelter 1976, Jeschke and Jambor 1981, Behl and Jeschke 1982, and Jeschke 1982), it was found that only ϕ_{oc}, β and Q were not affected.

Other values were underestimated by 11-14% and s_e was increased by 12%. It should be noted that the second term of the equation used to estimate Q (equation 2-21) was normally not greater than 10% of the first term. Therefore, the presence or absence of ϕ_{xc} does not affect Q greatly, providing that k_L remains unchanged.

This study also showed some evidence of re-translocation of tracer via the phloem (section 4.4.3 and 4.4.5). The re-translocated tracer seems to accumulate in the tip region rather than in the mature region of the root. Thus in a long-term kinetic study of tissue which includes the root tip, this translocated tracer may need to be taken into account and it complicates the simple model that has been used. Re-circulation of K^+ ions via the phloem to the root tip has also been suggested by Jeschke et al.(1983) using intact barley roots.

Bange (1977) suggested that the lag phase of tracer transport into the shoot was caused by an accumulation of ions into a fourth compartment before they were released to the vacuole and the xylem. This supported the finding of Pallaghy et al. (1970) of discontinuity of ion efflux in mature cells of corn roots under low external concentrations. In rice roots, there is no indication of any discontinuity of the efflux and, hence, the lag phase is not likely to correspond with the fourth compartment as suggested.

It has been shown in Fig. 4.12 that the major route of ions from the external solution to the xylem is via the symplast. If the tracer has to equilibrate with the cytoplasm of cortical cells, a lag phase of transport into the xylem is expected. As is shown Table 4.1, k_e of 0.83 hr^{-1} corresponds to a half time of short-term equilibration of about 0.8 hr. This finding supports Pitman (1971) that the lag phase is due to an equilibration of the tracer in the symplast.

4.6.1 Justification for neglecting longitudinal symplastic transport and apoplastic transport

Although pathways of ions transported into the non-labelled tip region of a root can be via both the symplast and the apoplast, it is not possible to separate these two in the

present study. However, if all transport is via the symplast, a value can be obtained from $\phi_c = (dY_c/dt)/s_c$, where dY_c/dt is 1.42×10^3 cph.hr^{-1} (see Table 4.2). Taking the average mass of the labelled tissue (1.17×10^{-6} kg/root) into account, ϕ_c is 0.04 m.equiv.kg^{-1}.hr^{-1}. This is not greater than 5% of the total radial transport into the xylem (ϕ_{cx}).

If there is apoplastic transport to the xylem, it should be most noticeable in the early stage of the uptake. This is because at this stage the specific activity of the cytoplasm is low. Thus, the appearance of tracer in the xylem in the 5-10 min loading experiments (section 4.4.4) can be used to give an upper limit of the apoplastic flux, ϕ_{ox}. The calculated value (i.e 0.05 m.equiv.kg^{-1}.hr^{-1}.) is not greater than 5% of the total symplastic transport (ϕ_{cx} = 1.15 m.equiv.kg^{-1}.hr^{-1}). The result suggests that, whether or not suberization of the endodermis is complete at the studied region of rice roots, the apoplastic transport can be neglected. This is consistent with other workers (Pitman 1971, Jeschke 1982, and Davis and Higinbotham 1976) who have assumed that this term is negligible but do not appear to have attempted to measure it directly.

4.6.2 The discrimination against Rb^+ in rice roots

The present study demonstrates that transport of ^{42}K and ^{86}Rb into the shoot is very much the same suggesting that both tracers are taken up across both the plasmalemma and the xylem boundary at the same rate. However, after the first hour when they are not significantly different, accumulation of tracer in the tissue is about twice as large for ^{42}K than ^{86}Rb. It is assumed that in the first hour period most of the tracer is in the cytoplasm and only a small fraction has moved into the vacuoles. Only after the vacuolar accumulation of tracers has become significant, do the two accumulation graphs diverge. This further supports the hypothesis that discrimination against Rb^+ occurs at the tonoplast. Under the conditions of these experiments, it is concluded that ^{86}Rb can be used as a tracer for K^+ as far as ion transport into the shoot and across the plasmalemma are concerned and that it underestimates ion

exchanges across the tonoplast.

A comparison between ^{86}Rb and K^+ accumulation in plant tissues was made by Jensen and Kylin (1980) in high-K^+ roots of cucumber, oat and wheat. Plants were grown in a labelled nutrient solution and analysed the amount of tracer in comparison to ion content in plant tissues. They found the same transport into shoots of oat and wheat for the two cations under high external concentrations (between 5-10 mM), but the accumulation of K^+(^{86}Rb) in the roots was greater than that of K^+. However, the result in cucumber showed no discrimination against ^{86}Rb, suggesting the possibility of varietal variation. They concluded that the site of the discrimination was at the tonoplast of cortical root cells. Behl and Jeschke (1982) used low salt barley roots and found greater values of ϕ_{oc}, ϕ_{co} and ϕ_{cx} for K^+(^{42}K) than K^+(^{86}Rb) and suggested that the site was at the delivery of ions to the xylem. In algal cells, such as *Cyanobacterium Anabaena variabilis*, Reed et al. (1981) found the influx of ^{86}Rb is smaller than that of ^{42}K and the difference is greater at low external concentrations.

The present result in rice roots together with those from the above workers seem to suggest that, the level of K^+ in the root itself determines the discriminatory properties in plant species. The dependence of Rb^+ uptake into roots on the internal K^+ status of the roots was reported by Pettersson and Jensen (1979) in sunflower roots and by Jensen (1981) in spring wheat roots.

A comparison of the results obtained in this study with those of other workers (Jeschke 1982 and Erlandson 1979) when K^{42} was used as a tracer, is shown in Table 4.9. It should be noted that work reported by Jeschke (1982) used roots whose tip had been discarded, while that of Erlandson (1979) used whole roots. Since there were no flux estimations made for wheat roots, a comparison can only be made between low salt barley and high salt rice roots. As can be seen, ionic fluxes and the rate of short-term exchange in low salt roots are much larger than those of high salt roots. This is not surprising, since ion efflux from the roots could reduce the rate of tracer build-up in the short-term component to some extent. The rate of long-term

Table 4.9

Showing K^+ fluxes and the internal content of plant roots, estimated by ^{42}K washout method.

HS - high salt roots
LS - low salt roots
WR - whole roots
PM - a portion of the mature root region
* - whole roots without the tip

k_e (hr^{-1})	k_L $\times 10^{-2}$	ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}	ϕ_{cx} ($m.equiv.kg^{-1}.hr^{-1}$)	s_c/s_o	Q_c ($m.equiv.kg^{-1}$)	Note
1.43	6.6	14.6	7.9	2.1	2.0	6.6		11.4	LS-* (a)
.83	.93	2.18	2.07	4.07	4.86	1.30	.30	9.2	HS-PM (b)
-	.90	-	-	-	-	-	-	-	HS-WR (c)

(a): Jeschke, 1982
(b): the present study
(c): Erlandsson, 1979

exchange in rice roots is similar to that of wheat, while that of barley appears to be about 7 times greater.

Since very few studies have been concerned with intact roots, the above comparison is somewhat inadequate for a generalization of the difference in ion movements between low salt and high salt roots. More work on varieties of plant species is required. In order to give an insight into the way in which movements of ions in intact roots differ from those in excised roots, the same study should be made in excised roots.

Chapter 5

Potassium Kinetics in Excised Roots

5.1 Introduction

As is known, one of the functions of the root is to accumulate salts in the cortex and transport them into the shoot via the xylem vessels. The transport of ions in excised roots is studied by observing the rate of ion exudation through the cut end of the root. This has been performed in corn and in barley by a number of workers (Anderson and House 1968, Smith 1970 and 1979, Parrondo and Smith 1976, Pitman 1971, and 1972b, Bange 1973, 1977, and Läuchli et al. 1978). In general, the transport showed a lag phase and the lag period varied depending on plant species.

In comparison to the efflux across the plasmalemma, transport into the xylem for K^+ and Cl^- appears to be smaller in low salt roots (i.e. grown in a medium without the studied ions) of barley (Pitman 1971, Jeschke 1977, and Behl and Jeschke 1982). In high salt roots (i.e. grown in a medium with the presence of the ions throughout plant life) of corn, the reverse was reported (Davis and Higinbotham 1976).

One advantage of using low salt roots is that they are able to absorb nutrients, especially the studied ions, at a greater rate than high salt roots due to salt deprivation. Another advantage is in the higher level of sugar in these roots than in high salt ones (Pitman 1976). This allows the roots to prolong their active state after being excised. However, after washing the roots for a period of time, a rapid fall of ion content in the roots was observed, even in the case when sugar was added into the root medium (Behl and Jeschke 1982). Although such roots have been widely used for flux studies, it can be argued that the results may not represent naturally growing roots. Experimental evidence showing an unusual increase in salt uptake after transferring low salt roots to a medium containing an ample concentration of the salt are from Pettersson (1975), Glass (1976), Drew and Saker (1978), Drew et al. (1984) and Lee (1982). So far, only the work by Davis and Higinbotham (1976)

appears concerned with high salt roots. More investigations in high salt roots are needed before one can generalise on the difference in ion uptake between low and high salt roots.

It has been argued previously that root tissue that contains the root tip is far from homogeneous and is, therefore, too complex for the simple mathematical model in chapter 2 to apply for it. At best the use of this model can only give average information about these wide range of cells. Despite this limitation, many previous workers as mentioned above have used excised roots in investigating ion kinetics in root tissue. For this reason such an analysis has been performed on data obtained from excised roots in this study so that comparison can be made between excised roots and intact roots (chapter 4) and also to allow comparison with the work of other investigators. As for intact roots, the suitability of ^{86}Rb as a tracer for K^+ ions was also tested with excised roots.

5.2. Experimental Materials

Seedlings of 7 days of age were used in work reported in this chapter. They were exposed to light one day prior to the experiments. Segments of roots between 0-10 mm and 10-20 mm from the tip were used and referred to as excised root tips and mature root segments, respectively. In some experiments, a K^+-free solution was used. The preparation of this solution has been described in the previous chapter (section 4.2.2).

Unless otherwise stated, the activities of labelled solutions used for uptake studies were 2 μCi/ml and for washout studies were 20 μCi/ml.

5.3 Experimental Methods

All of the experiments described as follows were carried out under the same conditions of growth. Aeration was supplied throughout the experimental periods by bubbling air through a small plastic tube. When an apparatus as shown in Fig. 5.1 was used, aeration was provided only in the middle chamber. Four roots were used in each observation.

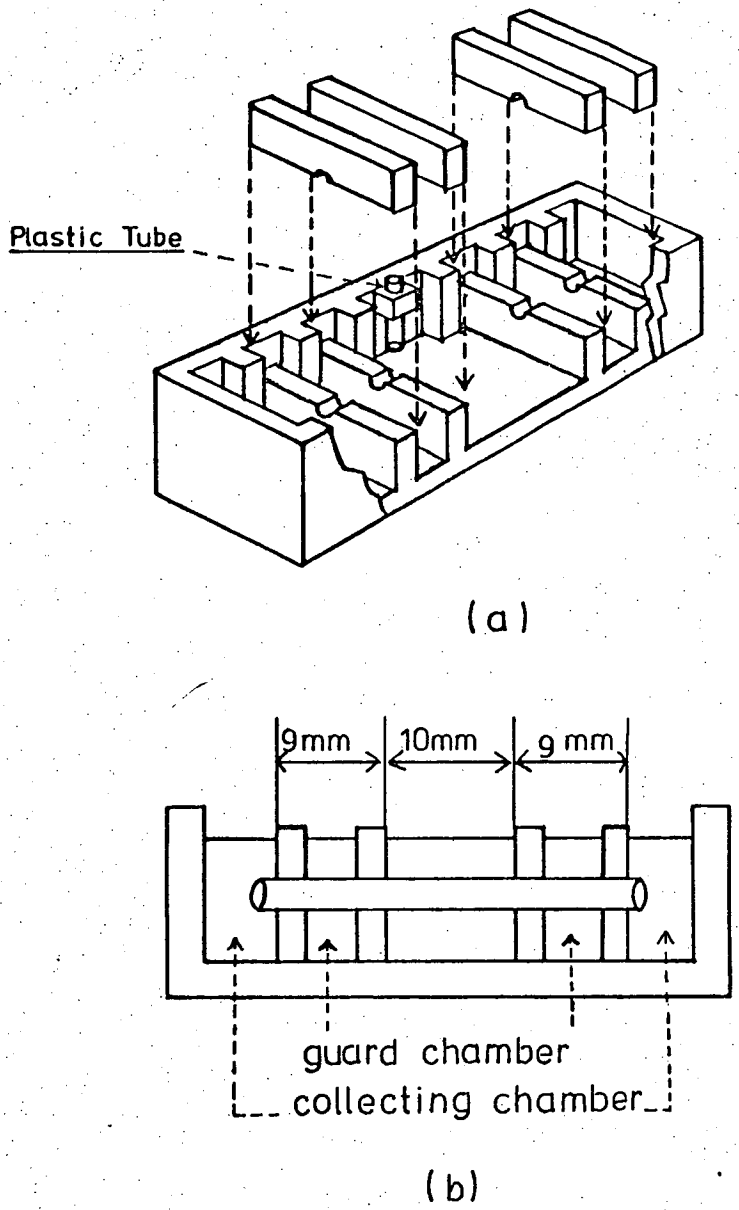


Fig. 5.1

An apparatus used for the measurements of ion loss through root cut ends and ion elution across the root surface. It was made from pieces of perspex with the size of 4.5x1.5x1.0 mm. The apparatus was divided into 5 compartments; the middle chamber, guard chambers and collecting chambers.

(a) Showing a three dimentional diagram of the apparatus. Two plastic tubes were attached to the side walls of the middle chamber; one for aeration facility and the other (opposite to the one shown in the diagram) for solution inlet.

(b) Showing a side view of a 30 mm root segment arranged in the apparatus. The segment was between 5-35 mm from the tip. The total length between each end of the guard chamber was 28 mm, with 10 mm in the middle chamber. This allowed 1 mm of the segment to emerge from the guard chambers to the collecting chambers.

5.3.1 Studies of tracer washout from root tissues

A range of 4-10 roots were used in each experiment. Excised roots and root segments were loaded with a labelled solution in a glass container soon after the excision. The ratio of the solution to tissue volume was about 150:1. At the end of loading, the solution was replaced with an amount of a 1x solution to rinse away the remaining isotope. Time taken for this rinsing was about 10-15 seconds. Tracer washout commenced immediately after this period.

The method of washout was the same as described in chapter 4, section 4.3.1, but the washout solutions contained ions lost both through the the xylem and by elution through the root epidermis.

In earlier experiments, washout was performed at room temperature (20° C) by using an automatic sample changing machine*, as shown in Plate 5.1. The small tube which penetrates through the washout container serves as an aerating tube during each washing period, and as a solution outlet at the end of washing. Since the result (see Fig. 5.5a) did not show any sign of discontinuity of efflux over 20 hrs of washing, later experiments were carried out manually under the same conditions as used for growth. Sample collection was made more frequently during the first 4 hour of washing. After this time, it was made over a longer period and sometimes was after an overnight washing. The overnight sample, normally, ran from 9 to 12 hrs. Each sample was dried slowly on a hot plate and counted. At the end of the experiment, root tissues were blotted and weighed before being crushed, dried, and counted. As before, the specific activity of the labelled solution was determined by diluting the solution to a known volume and 10^{-2} ml of this was dried and counted.

* see Pallaghy, 1968

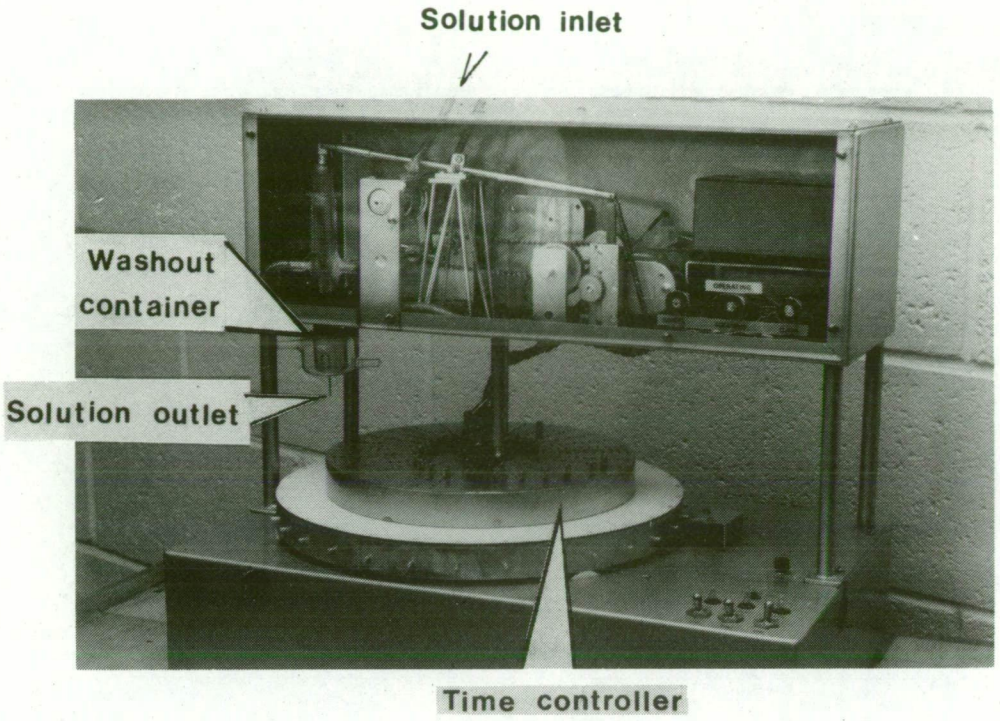


Plate 5-1. Automatic sample changing machine for tracer washout studies.

A non-labelled solution was fed into the washout container and at the end of each time interval the solution was ejected through a smaller metal tube, which also served as an aeration tube during washing. Glass containers were placed on the outer plate for sample collection, while the inner plate was the time controller.

5.3.2 Tracer measurements of K^+ transport through cut ends of roots

Roots were blotted gently and arranged in an apparatus as shown in Fig. 5.1. Note that the experimental chamber was bounded on each side by a 10 mm guard chamber. During preparation, the roots were handled above 40 mm from the tip to avoid damaging them. A water repellent grease was used to seal the end of each compartment. When transport into the root segments was to be studied, root excision was made at 5 mm and 35 mm above the tip, with a pair of sharp scissors, after they were arranged in the compartments. The portion of root in the middle chamber which was between 15-25 mm was labelled with a 2 μCi/ml ^{86}Rb labelled 1x solution. Time taken between the excision and the start of labelling was about 10 min. A 1x solution was used to fill in all the side chambers. When excised roots were to be studied, the excision was made at 20 mm from the tip and the portion between 0-10 mm was exposed to the labelled solution. This arrangement allowed about 1 mm of the root to emerge from the guard chamber into the collecting chamber.

To test whether there was any leakage between chambers, a neutral red solution was dropped into the guard chambers at the end of the experiments. Observations were taken for about 10-15 min. Tissues were blotted and weighed before being discarded.

5.4. Results

5.4.1 Tracer washout from root tissues

After washing in a non labelled solution, root tissues were blotted, weighed and counted. Graphs of tracer remaining in the tissues (Y) against time were constructed on a semi-logarithmic scale. Each data point was obtained by adding the activity remaining in the tissue at the end of a washing to the activity found in the previously washed out solution, for each time interval. Experiments were carried out in both excised root tip (i.e 0-10 mm from the tip) and mature root segments

(i.e. 10-20 mm from the tip).

Fig 5.2(a) and (b) show the time course of tracer remaining in the tissue of excised roots and root segments, respectively. In root segments, there appear to be three phases of tracer loss, the first taking place during the first 10 min, the second between 10 min and 3 hrs and the third after 4 hrs of washing. Note that most of the graphs of excised root tips did not show the three phases as clearly as those of root segments. The linear part of the graph started, sometimes, as early as 2 hrs after the washing commenced. This is consistent with the result obtained from a direct chemical analysis (section 5.4.5.2) and with the finding in barley root tip segments (Behl and Jeschke, 1982) when ^{86}Rb was also used as a tracer for K^+.

Extrapolation of the linear part of the graph to t=0 gives the total amount of tracer for long-term component (Y_L) and the slope is the rate constant for long-term exchange (k_L). The amount of the tracer for the short-term component (Y_s) can be obtained by subtracting the values of Y_L from Y and plotting the time course of the tracer remaining in the tissue on a semi-logarithmic scale (see the inset of Fig. 5.2). The slope is the rate of short-term exchange (k_s) and the extrapolation to t=0 gives the value of Y_s.

Note that in Fig. 5.2(b), the data were obtained manually and there was an overnight gap in data acquisition, while in Fig. 5.2(a) the samples were collected throughout the night by the automatic sample changing machine. In neither case was a discontinuity of tracer efflux observed as had been reported by Pallaghy et al. (1970) and Behl and Jeschke (1982). Also note that after about 20-30 hrs depending on the group of plants, there always appears a rapid loss of the tracer. This is similar to that reported in low salt barley roots by Behl and Jeschke (1982) which was suggested to be due to the cessation of metabolic-linked mechanisms.

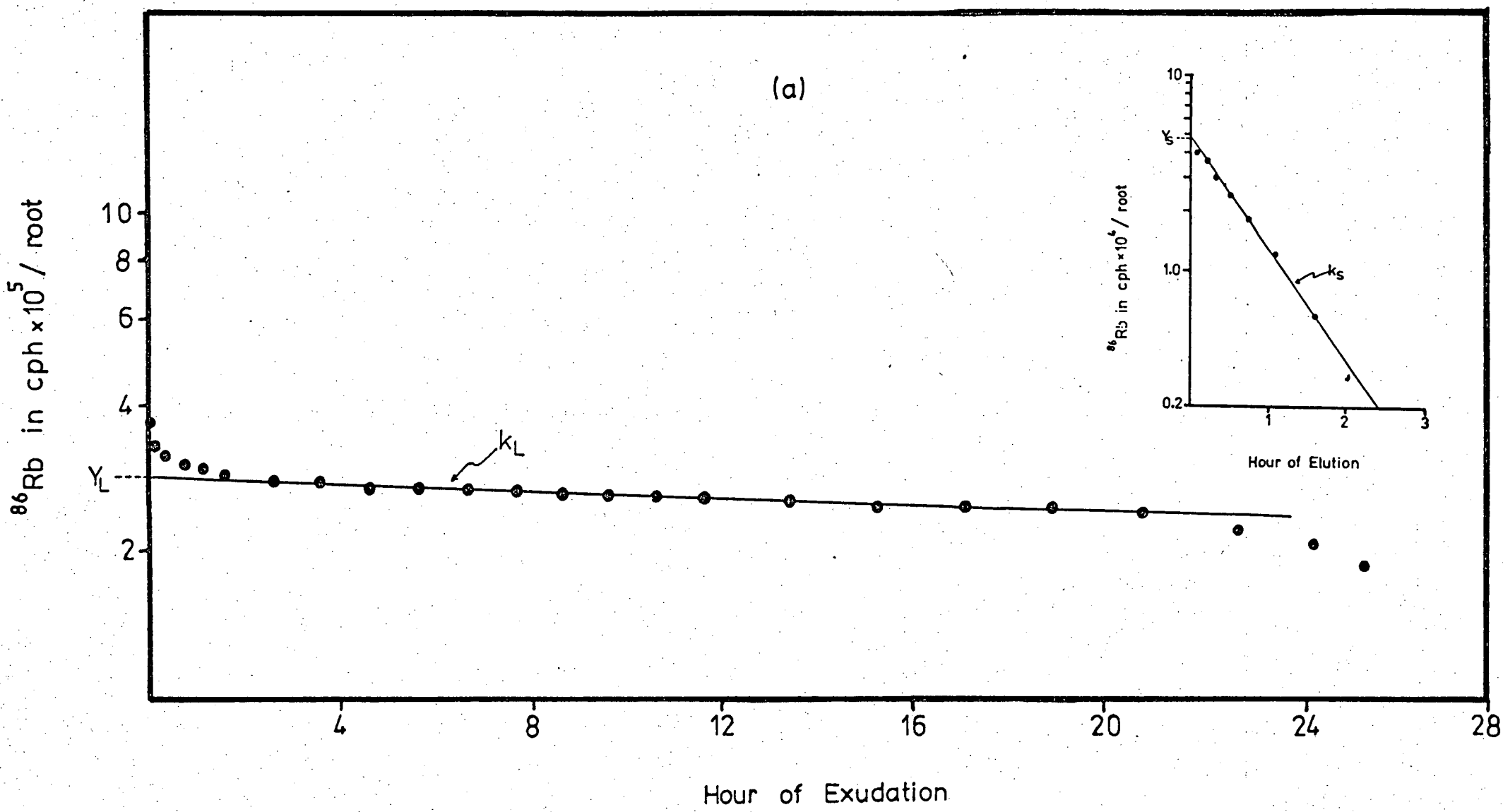
As can be seen from the equation (2-21) in section 5.5.1, k_L increases when the total tissue content decreases. During the quasi-steady state, Q decreases slowly and, hence, very little change in k_L can be observed. When a rapid fall in Q occurs, the slope of k_L would be expected to increase, as is seen

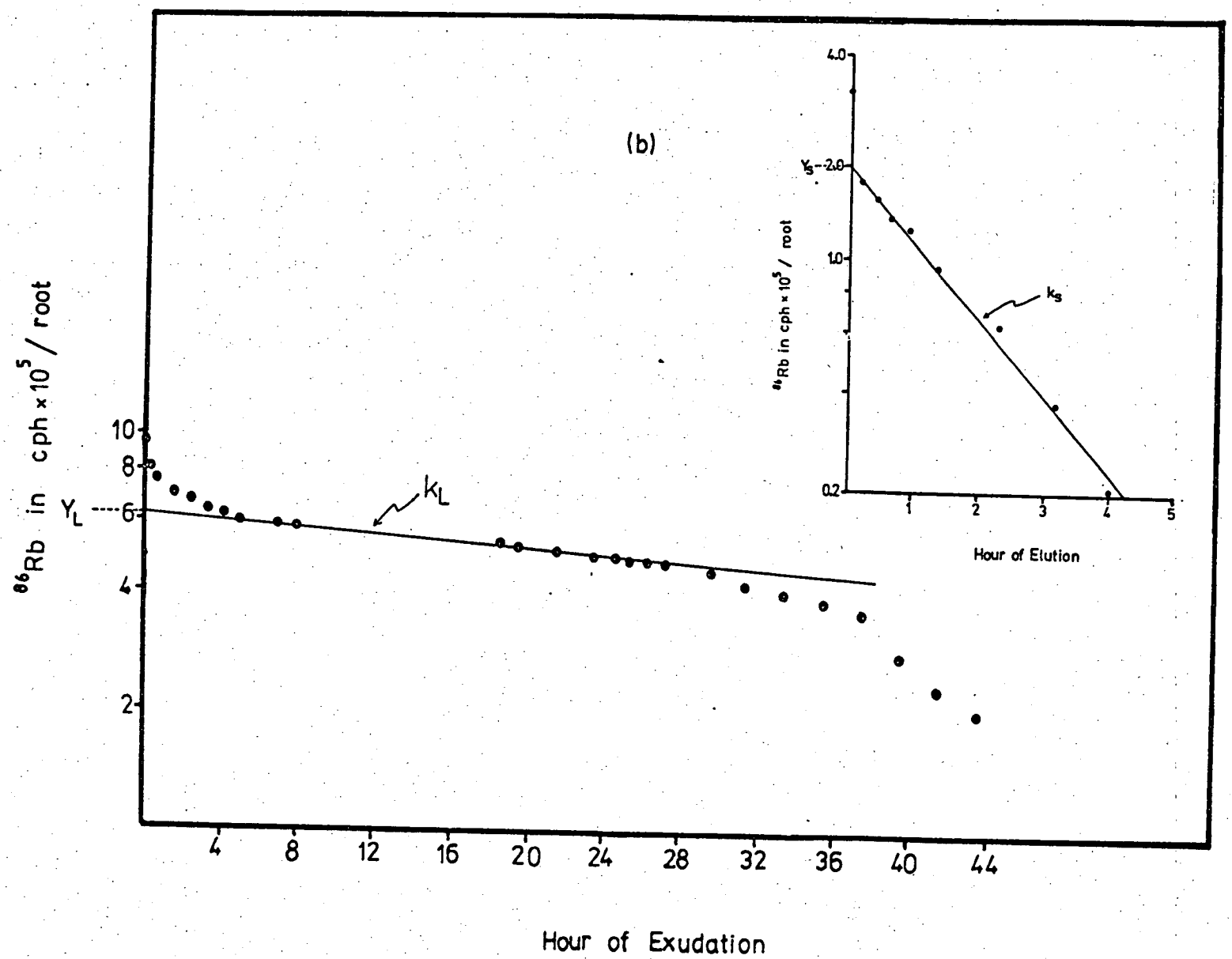
Fig. 5.2

Typical graphs of time course of tracer remaining in root tissues. Showing rates of long-term (k_L), short-term (k_s-see the inset) exchange and the amount of tracer in the vacuole (Y_L) and the cytoplasm (Y_s) after the tissues were loaded with a tracer for a period of time.

(a) When the experiment was carried out in excised root tips (0-10 mm from the tip) after 2 hr loading in a ^{86}Rb labelled solution. The washed out samples were collected by using an automatic sample changing machine (see Plate 5.1). Data was averaged from 10 roots.

(b) When the experiment was carried out in mature root segments (10-20 mm from the tip) after 5 hr loading in a ^{86}Rb labelled solution. The washed out samples were collected manually. Data was averaged from 11 roots.


(a)
^{86}Rb in cph x 10^{5} / root
10
8
6
4
2
Y_L
k_L
4
8
12
16
20
24
28
Hour of Exudation
^{86}Rb in cph x 10^{4} / root
10
Y_S
1.0
0.2
k_S
1
2
3
Hour of Elution


(b)
^{86}Rb in cph x 10^5 / root
10
8
Y_L
6
4
2
k_L
4
8
12
16
20
24
28
32
36
40
44
Hour of Exudation
4.0
Y_S 2.0
1.0
0.2
k_S
1
2
3
4
5
Hour of Elution

in the figures. This suggests that the change in k_L value after a long period of washing is a characteristic of excised tissues in general.

To test whether excised roots could discriminate against Rb^+ in favour of K^+ as reported for intact roots in the previous chapter, root segments were loaded in a labelled solution containing both ^{42}K and ^{86}Rb. The activity per ml solution was 10 μCi and 5 μCi, respectively. The period of loading was 5 hrs, the same for all experiments. Experiments were carried out in 3 batches of plants with 13 observations in total. Each observation contained 7-10 root segments. Due to the short half-life of ^{42}K (12.45 hrs), all activities in the washout solutions were corrected to the time when the experiment was started and adjusted further to the standard s_o value (0.73×10^{14} cph.mole^{-1} of K^+).

The graph of the time course of ^{42}K and ^{86}Rb remaining in the tissue, after all activities were adjusted to the same s_o value, is shown in Fig. 5.3. It was found that although the characteristics of the elution from the tissue for both tracers are similar, the amount of ^{42}K in the tissue at the end of washing is always greater than that of ^{86}Rb. In both cases, a rapid fall-off of tracer content was observed after about 20 hrs of washing.

A series of k_s, k_L, Y_s, and Y_L values for excised roots and root segments obtained from graphs as depicted is shown in Table 5.1. In order to compare the results from each group of root tissue, Y_s and Y_L are expressed in m.equiv.kg^{-1} of tissue fresh weight by taking into account the specific activity of the medium during tissue loading. It appears that Y_L is greater than Y_s for all cases. At a shorter period of loading time (T), Y_s and Y_L are smaller.

Table 5.1(a) and 5.1(b) show that each of the values of k_s, k_L, [illegible] are smaller for excised root tips than for more mature root segments. Table 5.1(b) and 5.1(c) show that Y_L, k_s and k_L are smaller when ^{86}Rb is the tracer than when ^{42}K is used, although the two tracers give similar values for Y_s.

In order to estimate ion fluxes and the internal content, it is important to know whether the root tissues were at

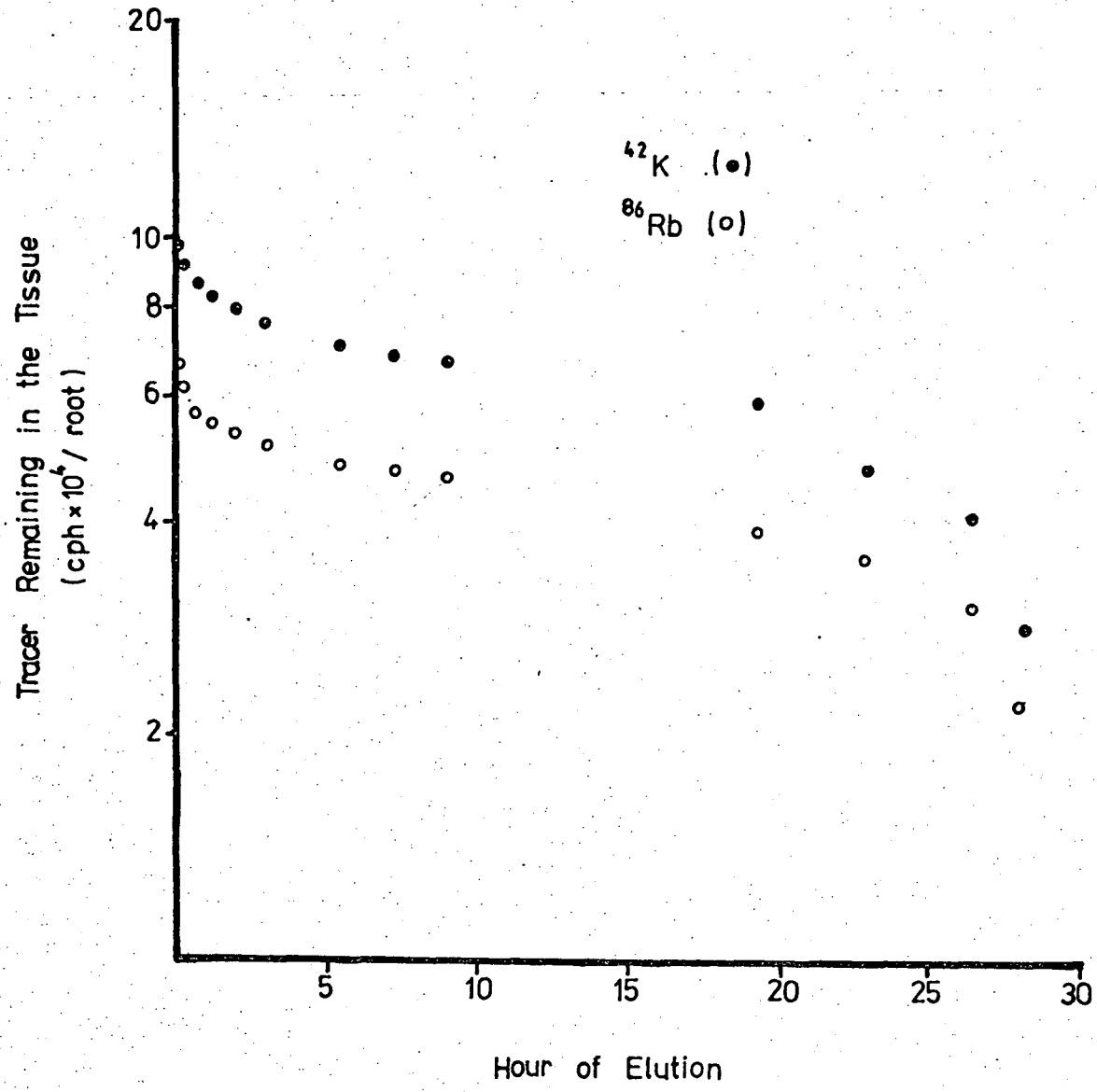


Fig. 5.3

A comparison between ^{42}K (●) and ^{86}Rb (o) washout from mature root segments, after 5 hour isotope loading. All activities were adjusted to the same specific activity of the medium.

Table 5.1

A series of k_s, k_L, Y_s and Y_L obtained from washout experiments after root tissues were labelled in a ^{86}Rb containing solution for different periods of time (T).

(a) Root tips (0-10 mm)

Exp. no	T	k_s	k_L	Y_s	Y_L
		10^{-2}			
1	2	1.15	.99	.26	1.70
2		.70	.95	.18	.67
1	5	.88	2.50	.83	3.02
2		1.08	1.50	1.10	2.01
3		.53	1.68	.66	3.71
4		.52	.93	1.17	2.46
5		.46	1.06	.60	2.52
6		.51	1.16	.53	2.14
$\bar{X}$ ± S.E.		.73 ± .10	1.35 ± .19	.67 ± .13	2.28 ± .32

(b) Root segments (10-20 mm)

Exp. no	T	k_s	k_L	Y_s	Y_L
		10^{-2}			
1	2	.89	1.72	.17	.52
2		.53	.63	.13	.41
1	5	.56	1.11	.40	1.28
2		.68	1.32	.19	.82
3		.91	1.42	.13	.85
4		.71	1.84	.61	2.65
5		.50	2.28	1.44	2.13
6		1.08	1.98	.97	1.24
7		1.12	2.18	.23	1.23
8		1.16	2.11	.26	1.01
9		1.14	1.94	.29	1.71
10		1.57	1.72	.56	1.08
11		1.04	2.42	.44	1.18
12		1.07	3.39	.17	.73
13		1.00	3.81	.10	.68
14		1.09	1.99	.11	.58
15		.95	2.06	.11	.49
16		1.02	3.34	.27	.63
17		.49	2.25	.17	.73
18		1.13	1.51	.21	.79
19		.90	1.50	.12	.52
20		.65	1.41	.11	.55
21		1.71	2.78	.20	.52
22		1.01	1.98	.16	.40
23		.63	2.56	.47	.65
24		.71	1.32	.38	.63
$\bar{X}$ ± S.E.		.93 ± .06	2.02 ± .14	.32 ± .06	.92 ± .11

T = hr
k_s and k_L = $(hr)^{-1}$
Y_s and Y_L = $m.equiv.kg^{-1}$

Table 5.1(c)

A series of k_s, k_L, Y_s and Y_L obtained from washout experiments of root segments (10-20 mm from the tip) when ^{42}K was used as a tracer.

Note T in hr, k_s and k_L in $(hr)^{-1}$ and Y_s and Y_L in $m.equiv.kg^{-1}.hr^{-1}$.

Exp. no	T	k_s	k_L	Y_s	Y_L
			10^{-2}		
1	5	.68	1.38	.34	2.33
2		.62	1.46	.37	1.77
3		.94	1.41	.33	2.39
4		.69	1.41	.34	2.06
5		.77	1.71	.59	1.74
6		.51	1.89	.24	.48
7		.92	1.48	.26	.81
8		.65	1.35	.17	.75
9		.48	1.37	.19	.80
10		.87	1.28	.21	.54
11		.85	1.95	.25	.48
12		.51	1.56	.65	.83
13		.62	1.30	.55	.87
$\bar{X}$ $\pm$ S.E.		.70 $\pm$ .04	1.50 $\pm$ .06	.34 $\pm$ .04	1.22 $\pm$ .20

steady state conditions and the rate at which ions are transported into the xylem. These were investigated in the following sections.

5.4.2 K^+ content in aged root tissues

To test the state of root tissues under investigation, the amount of K^+ content in excised root tissues was analysed after they were aged in a culture solution for various periods of time. Only if there is no net loss or gain of K^+, are the tissues at steady state (i.e. $\phi_v = 0$). The general method employed for this study was by ageing segments of excised root tips and root segments in a large volume of 1x solution and washing them in a K^+-free solution (section 4.2.2) for 30 minutes. They were then blotted and weighed. The method for extracting K^+ ions and analysing the content was the same as described in chapter 4 (section 4.3.2). Samples of 5-10 segments were taken from the solution at a time. Note that the culture solution was changed to a fresh one every 10-12 hours to avoid an undue change in pH value or salt concentration of the solution. The experiments were carried out using 3 batches of plants, with a total of 6 observations.

The result is shown in Fig. 5.4. The content at 0 hour was determined from freshly excised roots from whole plants which remained attached during the 30 min wash. It shows that K^+ contents in both groups of tissue fall rapidly at the beginning of ageing. The fall, however, becomes much slower between about 5-30 hrs of the ageing with a slightly greater rate for root segments than excised roots. After being aged for about 45 hrs, there appears a fall of the content in both tissues. Note that this is more pronounced in mature root segments than in excised root tips.

It is worth pointing out that K^+ content in excised root tips is always greater than for the segments. Although K^+ content in the tissues are changing with time, the slow change is taken for the rate of change during a quasi-steady state of the tissue. During the period from 5-30 hours when the K^+ content

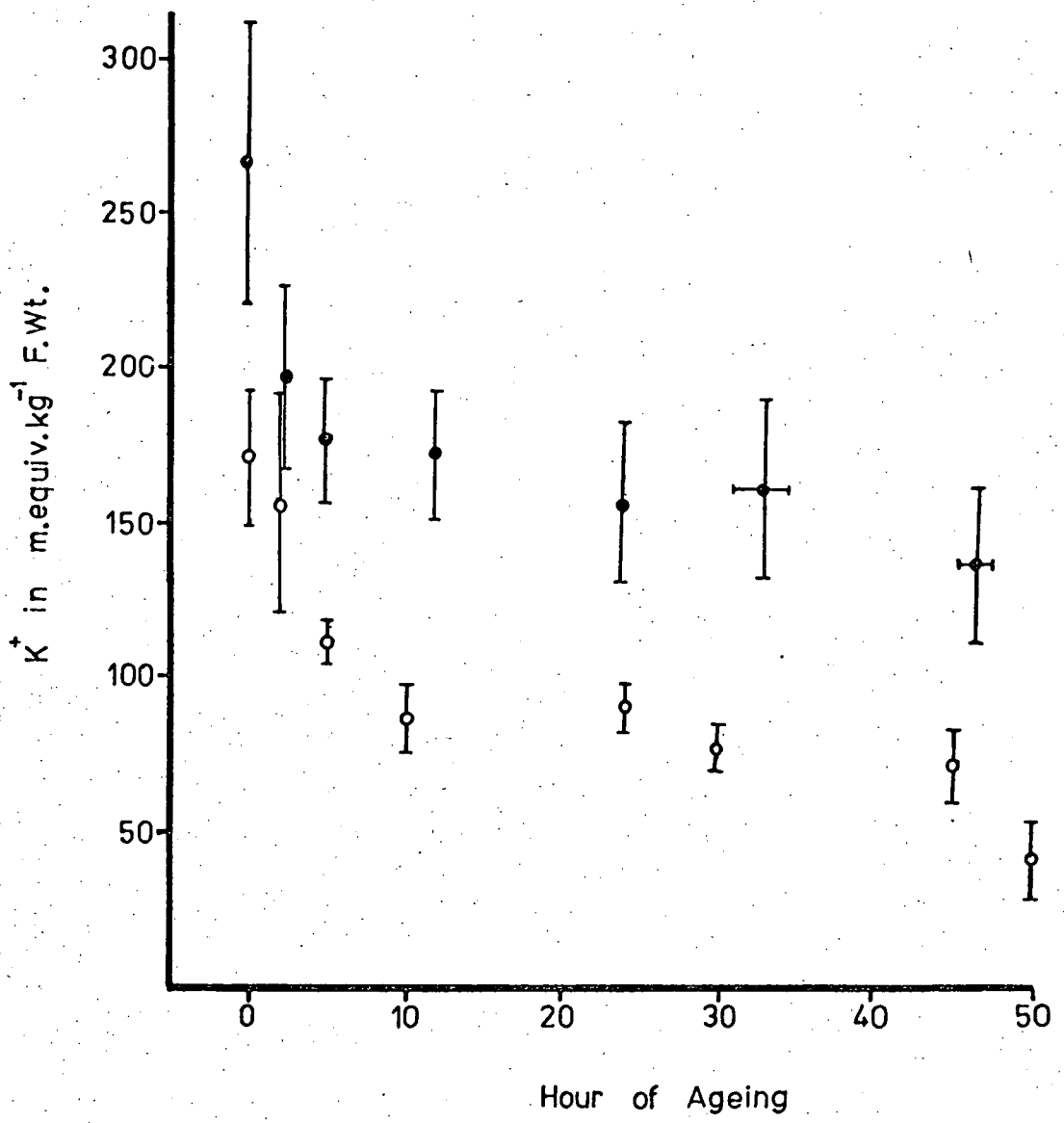


Fig. 5.4

Time course of K^+ concentration in root tip segments (●) and mature root segments (o) after they were aged in a ix solution under the same conditions as growth, with a continuous aeration, for several time intervals. The limit denotes the standard error of the mean from 6 (●) and 4 (o) observations.

is changing only slowly the standard tracer methods for flux study can be applied. Despite the large variability of K^+ content values (due to the fact that different plants were used in each analysis), there appears to be a small fall during this period. This is estimated to be about -1.00 $m.equiv.kg^{-1}.hr^{-1}$. The average Q taken during the slow change is 87 ± 5 $m.equiv.kg^{-1}$ and 174 ± 9 $m.equiv.kg^{-1}$ for mature root segments and excised root tips, respectively.

5.4.3 Tracer studies of K^+ transport through cut ends of roots

The measurements in this section were based on the assumption that the amounts of tracers found at the cut end, after the tissue was loaded, were those transported through the xylem vessels. Three experiments were carried out for comparison. Two groups of roots were excised; one at 20 mm and the other at 35 mm from the tip. The former was labelled between 0-10 mm and the latter between 15-25 mm from the tip. Experiments in the latter were separated into two; one without the 0-5 mm root tip and the other with the tip (see inset in Fig. 5.5a) so that the effect of the tip on tracer transport into a mature cell region can be studied. In preliminary studies, the exudates from each end were separated. The results, taken from 2 observations, showed that the amount of tracer lost through the lower cut end was about 20% of that from the upper end (i.e at 35 mm). This differs from Behl and Jeschke (1981b) who found that tracer loss through the apical cut end (8 mm from the tip) of barley roots was negligible.

The total exudation at various time intervals is shown in Fig 5.5(a). Since the specific activity of the xylem sap is not known, the exudate is expressed in $cph.kg^{-1}$, relative to the labelled portion of the root. In all cases, the distance travelled by ^{86}Rb from the entry site to the cut ends is 10 mm. It was found that the tracer could be detected very soon, after about 15 min of tissue loading. In contrast to the cases in which mature tissue is loaded, there is a lag phase in the exudation when the tracer enters the root tip portion, for about 3 hours after the loading has commenced.

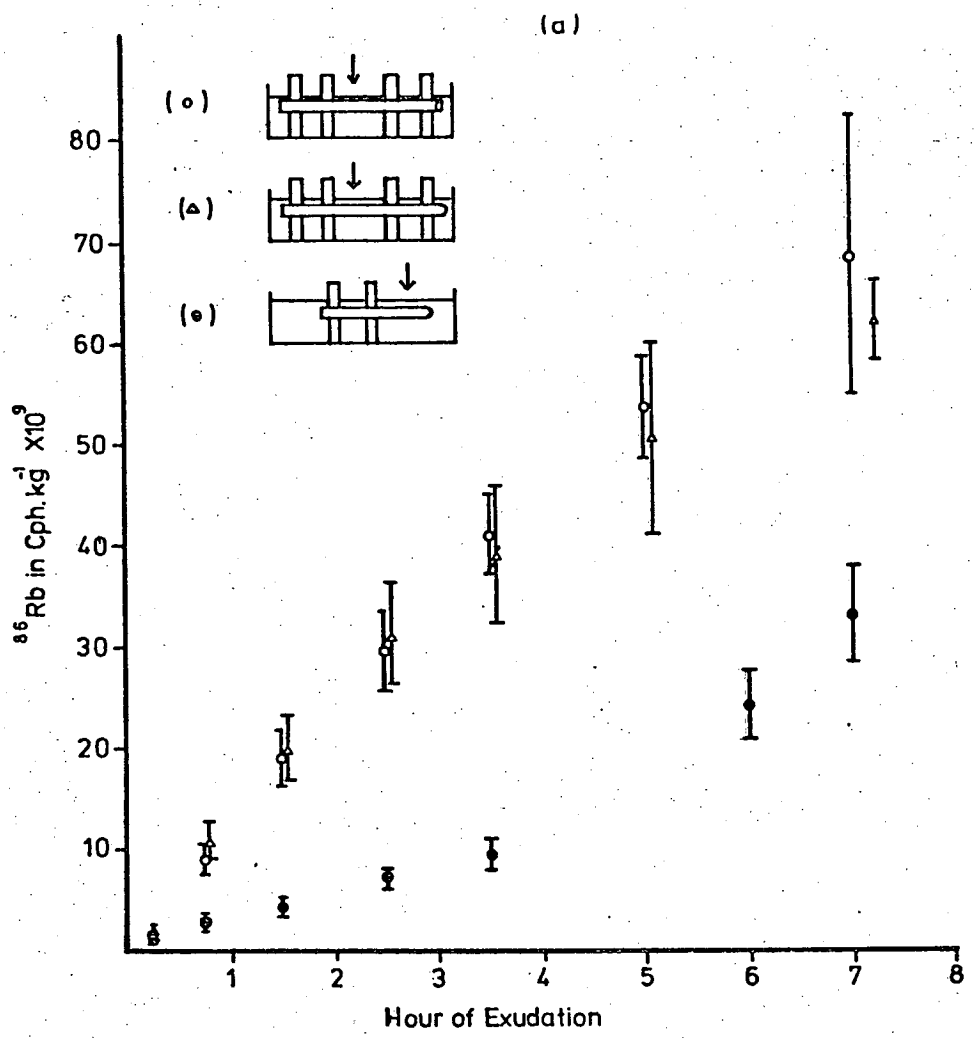


Fig. 5.5(a)

Time course of ^{86}Rb exudation in activity per kilogram fresh weight of the labelled tissue when the labelling was made between 0-10 mm (●), 10-20 mm (O) and 15-25 mm (Δ) from the tip. The limit is the standard error of the mean of 4 observations, 16 roots in total. All activities were adjusted to the standard s_o (= 0.73×10^{14} cph.mole^{-1} K^{+}).

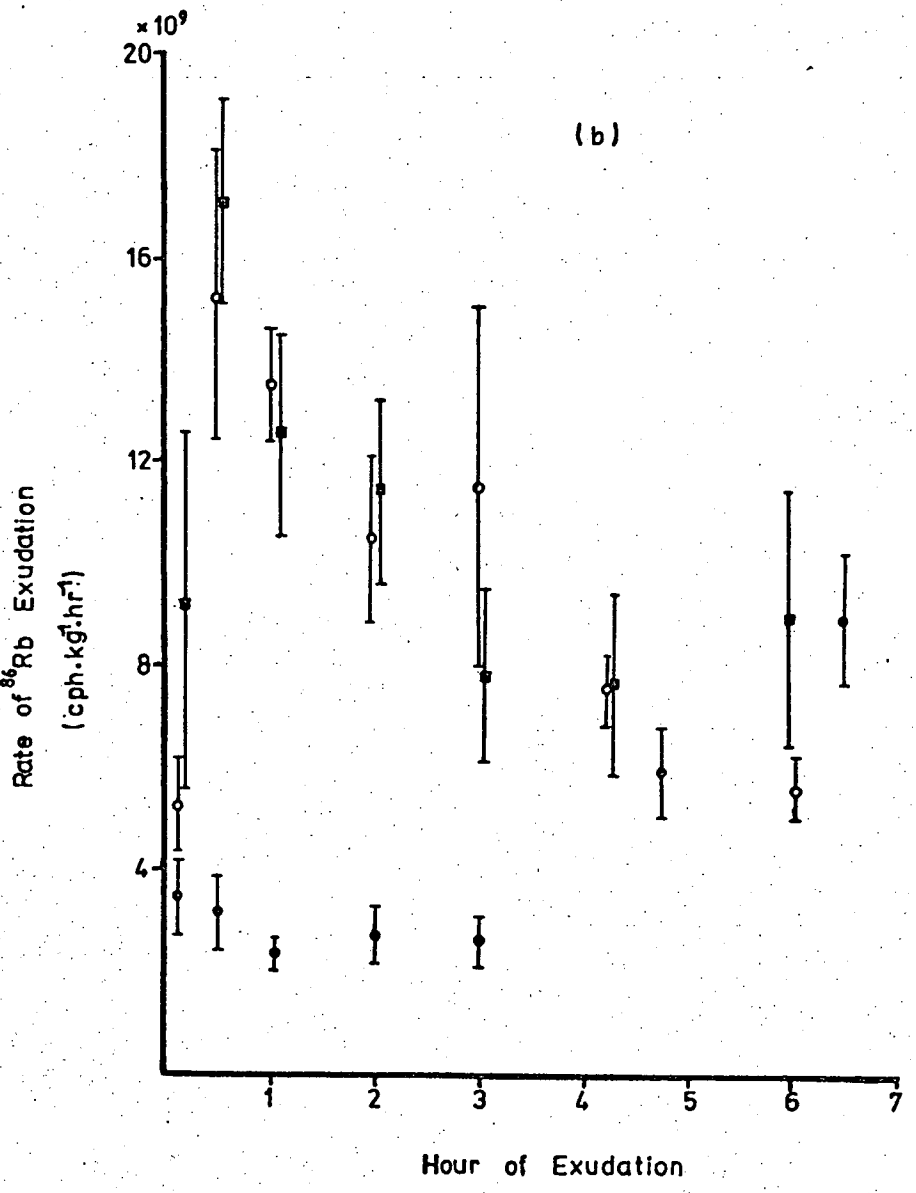


Fig. 5.5(b)

Experimental results in Fig.(a) were plotted as the rate of ^{86}Rb exudation, in $cph.kg^{-1}.hr^{-1}$, when tissue labelling was made between 0-10 mm (●), 10-20 mm (○) and 15-25 mm (△) from the tip.

In the case of transport into mature cells, the time course of the amount of tracer found at the cut ends of roots appears to be independent of the presence of the 0-5 mm tip region. If the amounts of tracer found at the cut ends were from symplasmic transport into the xylem vessels, a lag phase of the transport due to equilibration of the tracer in the cytoplasm is expected. The non-appearance of the lag phase contrasts with the finding for intact roots and this will be discussed later.

Fig. 5.5(b) compares the rate of exudation between these experiments. It appears that the exudation rate from the mature cell region is large during the first 30 minutes and decreases steadily thereafter. The rate remains fairly constant beyond 3 hrs of loading. In contrast, the rate of exudation when the root tip is loaded is constant for the first three hours and is much less than when mature tissue is loaded. However, the rate for tip loading increases greatly after 3 hours and becomes comparable with that for mature tissue. The fairly constant rate between 1-3 hrs is due to the delay.

It should be noted that a lesser capability to transport ions into the xylem in the mature part compares to the tip region was reported earlier in corn roots by Smith (1970), and was confirmed by Smith and Majeed (1981).

To test whether transport into the xylem of K^+ ions can be studied by the use of ^{86}Rb, experiments were carried out in root segments excised between 5-35 mm from the tip and labelled with both ^{42}K and ^{86}Rb. The activities for the tracers in the labelled solution were 5 μCi/ml and 2 μCi/ml, respectively. As before, the exudate through both cut ends were combined. Activities of both tracers were adjusted to the standard value (i.e. 0.73×10^{14} cph.mole^{-1} of K^+).

The results are shown in Fig. 5.6. As was observed previously, the rate of exudation for ^{86}Rb increases soon after the excision and decreases some time later. The magnitude is significantly greater for ^{86}Rb than ^{42}K between 30 min and 4 hrs of loading. This is probably because freshly excised roots retain some of the characteristics of intact roots for some time after excision (i.e. 0-4 hrs) and during this time ^{42}K is absorbed into the vacuole at a greater rate (see section

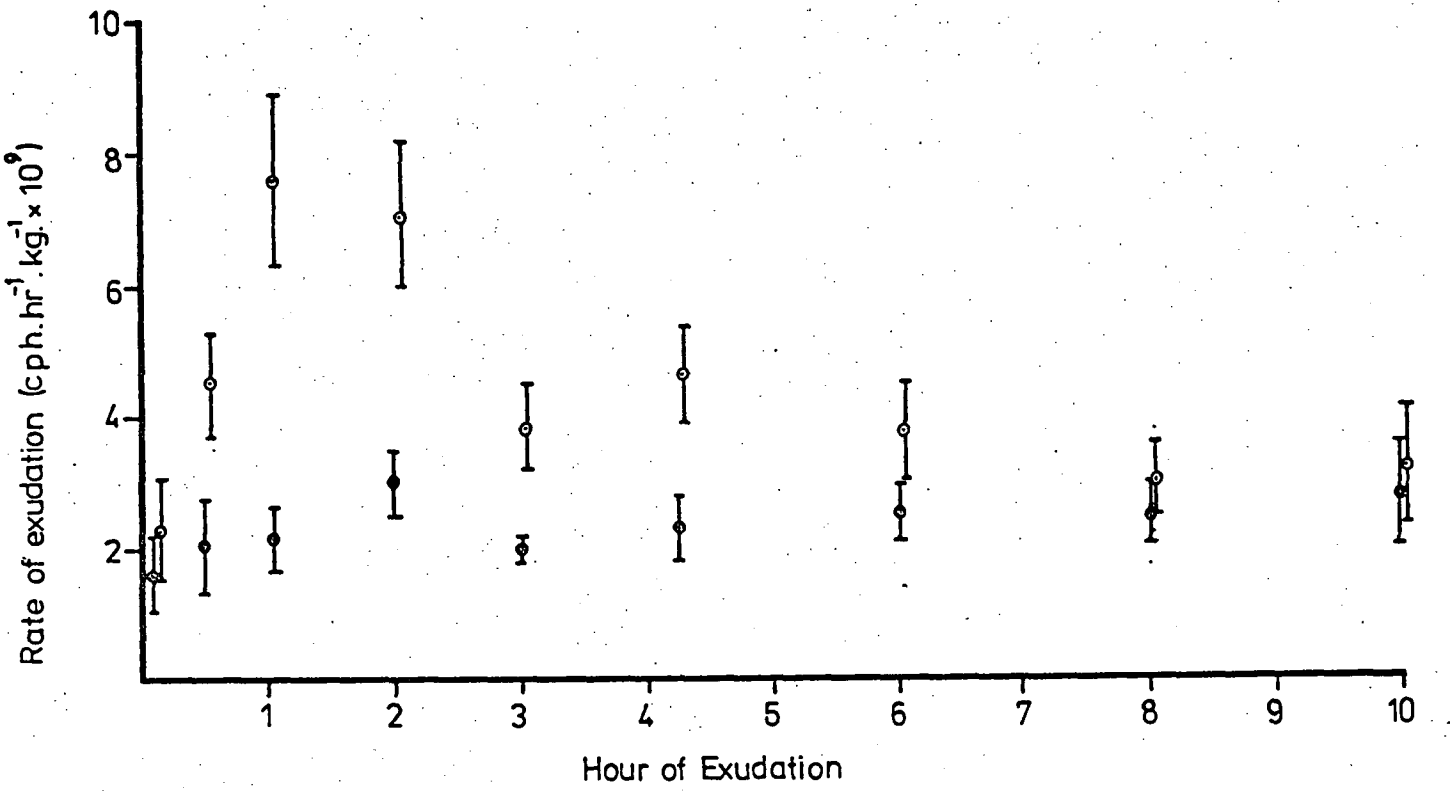


Fig. 5.6

A comparison between the rate of ^{42}K (●) and ^{86}Rb (○) exudation through root cut end at 35 mm from the tip of 5-35 mm root segments when tissue labelling was made between 15-25 mm from the tip. The unit is expressed in relation to the mass of the labelled portion. The limit denotes the standard error of the mean of 6 observations, 24 roots in total.

$s_o = 0.73 \times 10^{14}$ cph.mole^{-1} K^+.

4.4.3). During this time, s_c in the cytoplasm remains lower for ^{42}K than for ^{86}Rb and hence the flow of ^{42}K to the xylem is less than of ^{86}Rb. At a later stage, the tissue is less active and, consequently, the transport across the stele for both tracer is similar. This was found to be the case after about 6 hrs when both rates are not significantly different. On average between 3-10 hrs, the exudation rate (dY_x/dt) is 2.44×10^9 cph.hr^{-1}.kg^{-1} for ^{42}K and 3.10×10^9 cph.hr^{-1}.kg^{-1} for ^{86}Rb. These values will be utilised later in section 5.5.1.

5.4.4 Tracer measurements of ion accumulation in root tissues

It has been demonstrated in the previous section that tracer transport to the xylem differs depending on whether or not the root tip is loaded. There is a much longer lag phase when the tip is loaded. The study in this section is to observe the characteristics of tracer accumulation into both mature and tip region of the root. Experiments were carried out using excised roots and root segments. Both groups of tissues were loaded in a ^{86}Rb labelled solution with an activity of 2 μCi/ml. At selected intervals, a group of 3-5 segments were transferred to a 1x solution, to wash off the surplus isotope, for 30 min. They were blotted and weighed, then crushed and evaporated slowly on a hot plate before being counted.

The amount of $K^+(^{86}Rb)$ found in the tissue at the end of loading was expressed in m.equiv.kg^{-1} of root fresh weight and plotted against loading time, as shown in Fig. 5.7. Due to the 30 min wash, most of the tracer found in the tissue will be that which has accumulated in the vacuoles. There appears two rates of $K^+(^{86}Rb)$ accumulation in excised roots with the root tip; a greater rate appearing at the beginning of the loading and a smaller one appearing after about 1.5 hours. Note that this greater rate occurs during the lag phase of transport into the xylem (Fig. 5.5a). Since the lag phase is due to the time taken to equilibrate the tracer ions in the cytoplasm (chapter 4), the accumulation at the beginning could be that to the cytoplasm of the young cells rather than to the vacuoles. Since tracer was still found after 30 min of washing this may indicate that 30 min

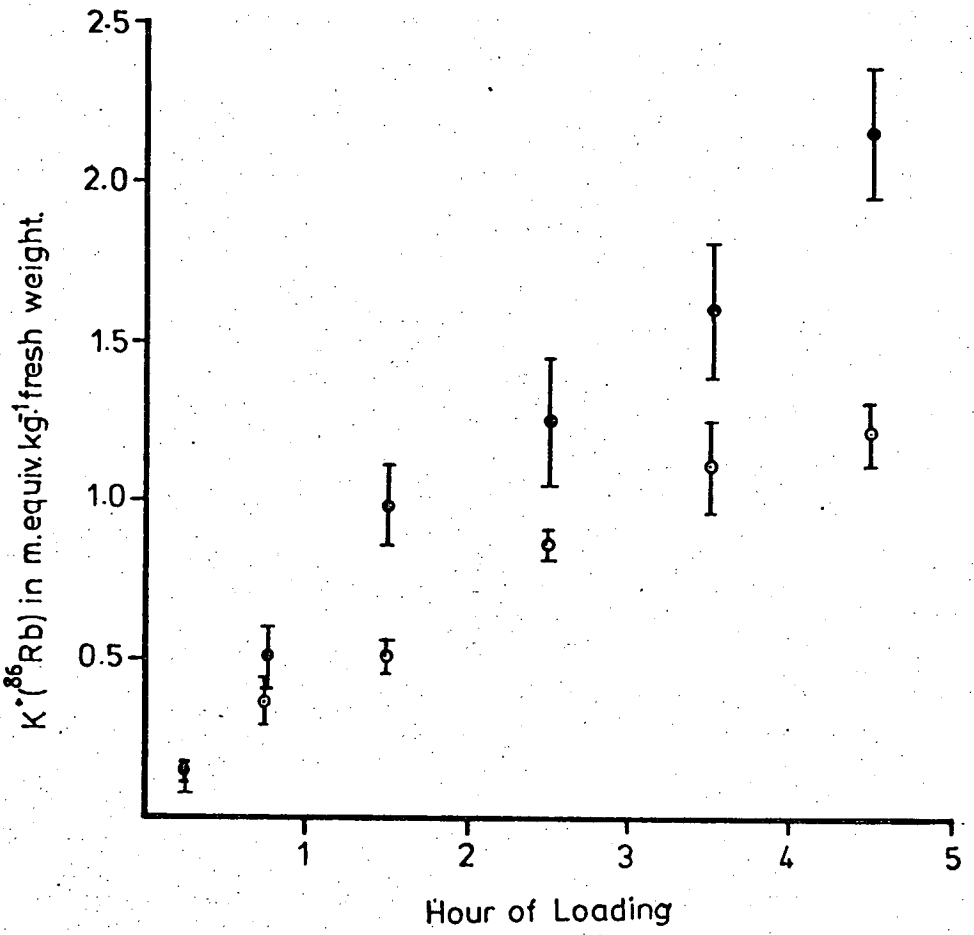


Fig. 5.7

Time course of tracer studies of K^+(^{86}Rb) accmulation in root segments between 0-10 mm from the tip (●) and 10-20 mm from the tip (○). The study was made under the same conditions as growth with a continuous aeration. The limit is the standard error of the mean of 4 observations.

wash was not long enough to get rid of most of the cytoplasmic content. In other words, non-vacuolated cells at the tip region are able to retain ions more easily than mature cells. Also note that the accumulation rate in the tissue becomes smaller when transport to the cut end becomes prominent.

When mature root tissue is loaded, the rate of K^+(^{86}Rb) accumulation is near-constant for 4 hrs at least. Interestingly, this contant accumulation rate occurs simultaneously with the continuous transport to the cut end (Fig. 5.5a), suggesting that any lag phase both for transport and accumulation is much smaller for the mature segments than for root tip segments.

It is worth pointing out that the accumulation is greater in excised roots than root segments at any loading time.

5.4.5 A direct estimation of K^+ fluxes from chemical assay method

In order to compare the net uptake of ions into roots ($\phi_{oc} - \phi_{co}$) and the transport into the xylem with the results obtained from washout studies (section 5.5.1), a direct chemical assay method for measuring K^+ fluxes across the plasmalemma and into the xylem was designed. This was performed by arranging excised roots in the apparatus (Fig. 5.1) as described in section 5.3.2, except all side chambers were filled with a K^+-free solution and the middle chamber was filled with either the 1x solution or the K^+-free solution (see below). At time intervals, the exudate was collected and analysed for K^+ ions, using an Atomic Absorption Spectrophotometer (section 3.3.2.1). The following is to describe the study using root segments (5-35 mm from the tip) and excised roots (0-20 mm from the tip).

5.4.5.1 In mature root segments

In a preliminary study, root segments of 5-35 mm were arranged in the apparatus and the middle chamber was filled with a 1x solution. The exudate from both cut ends were collected

separately. It was found that K^+ exudate at the lower cut end of the root (i.e. at 5 mm) was between 17-20 % of that at the upper end (i.e 35 mm) at the end of 6 hrs. That at the upper end included K^+ that originated in the medium bathing 15-25 mm portion of the root and also included K^+ lost from root cells for the entire length of the segment. Consequently, further experiments were designed to distinguish the K^+ from each source. It was carried out in paired experiments; one with the 1x solution in the middle chamber, and the other with the K^+-free solution. At selected intervals of time, the exudate was analysed for K^+ ions. At the same times, K^+ eluted into the K^+-free solution was also analysed, so that the rate of ion elution (ϕ_{co}) from the portion of root can be obtained. The equation for ion absorption into the root and transport into the xylem for the paired experiments are

$$(\phi_{oc})_1 - \phi_{co} = \phi_v + (\phi_x)_1 \qquad (5\text{-}1)$$

and

$$(\phi_{oc})_o - \phi_{co} = \phi_v + (\phi_x)_o \qquad (5\text{-}2)$$

where $\phi_v = (\phi_{cv} - \phi_{vc})$ and $\phi_x = (\phi_{cx} - \phi_{xc})$. The subscripts refer to the experiment using the 1x solution ($_1$) and the K^+-free solution ($_o$). Since the K^+-free solution in the middle chamber was changed frequently, the re-absorption, $(\phi_{oc})_o$, of ions which were eluted from the tissue was assumed to be negligible. Therefore, the difference between two exudation rates is ϕ_{oc}- the influx across the plasmalemma. ϕ_v is determined by $\phi_{co} + (\phi_x)_o$ when roots are exposed to the K^+-free solution.

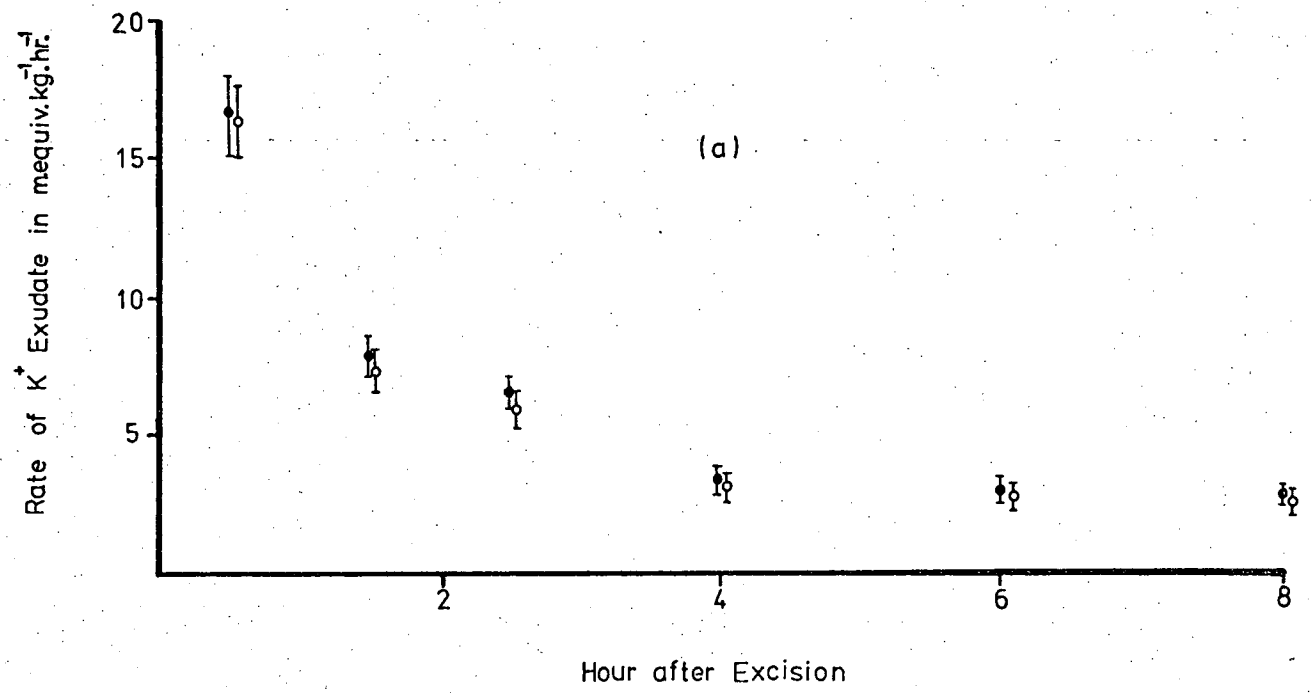
Fig.5.8(a) shows the rate of K^+ loss through root cut ends, expressed in m.equiv.kg^{-1}.hr^{-1} relative to the mass of the whole root length of both experiments. It appears that ϕ_x is greater at the beginning due to, possibly, the internal osmotic pressure at the time of the excision. This exudation rate rapidly reduces during the first hour, and becomes steady after 4 hrs. Although $(\phi_x)_1$ and $(\phi_x)_o$ are not significantly different throughout the 8 hr period, the former tends to be slightly greater. Due to the similarity of the exudation rates, it is

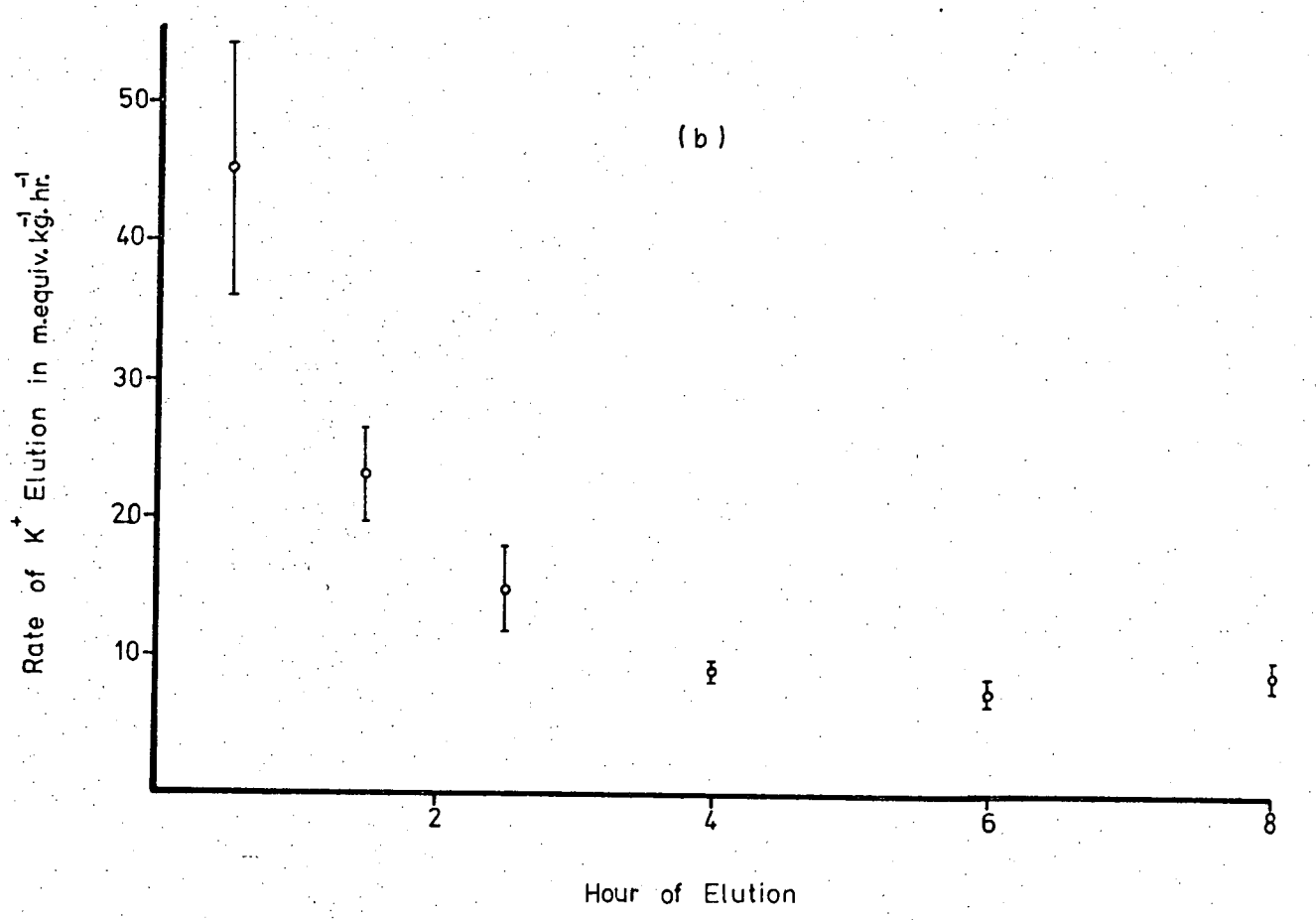
Fig. 5.8

(a) A comparison of the rate of K^+ loss through 35 mm cut end of 5-35 mm root segments when a portion between 15-25 mm from the tip was bathed in a 1x solution (●) and a K^+-free solution (○). The rest of the segment was in the K^+-free solution.

(b) Showing the rate of K^+ elution in $m.equiv.kg^{-1}.hr^{-1}$ from a portion between 15-25 mm from the tip of 5-35 mm root segments into a K^+-free solution.

The limit shows the standard error of the mean of 6 (a) and 4 (b) observations. Each observation contained 4 roots.


Rate of K+ Exudate in mequiv.kg.-1.hr.-1
20
15
10
5
(a)
2
4
6
8
Hour after Excision


Rate of K+ Elution in m.equiv.kg.-1.hr.-1
50
40
30
20
10
(b)
2
4
6
8
Hour of Elution

suggested that ϕ_{oc} is small in comparison to ϕ_x and the ions found at the cut end is mostly derived from the vacuoles. This result indicates that the root tissue is losing K^+ after the excision. This is confirmed when K^+ content in aged root segments were determined, in next section.

Fig. 5.8(b) shows the rate of K^+ elution in relation to the mass of a 15-25 mm root portion. There are three rates, the first occurring during the first hour, the second during the next 2 hrs and the last after 4 hrs of the experiment. The constant ϕ_{co} which is averaged between 4-8 hrs is 8.3 m.equiv.kg^{-1}.hr^{-1}.

Note that this elution rate is about 2.8 times greater than ϕ_x. The result leads to a speculation that the above ϕ_x could be overestimated, due to the elution from 1 mm of root emerging from the guard chambers into the collecting chambers. After taking this into account, $(\phi_x)_o$ becomes about 1.3 m.equiv.kg^{-1}.hr^{-1}, and ϕ_v is 9.6 m.equiv.kg^{-1}.hr^{-1} (= 8.3 m.equiv.kg^{-1}.hr^{-1} + 1.3 m.equiv.kg^{-1}.hr^{-1}).

5.4.5.2 In excised root tips

Experiments were carried out in a portion of root 0-10 mm from the tip using the same method as above. The results are shown in Fig. 5.9(a) and (b). As appeared in mature root cells, ϕ_x decreases continuiously from the beginning until about the fourth hour of the experiment when it becomes fairly constant. Comparing between the two fluxes, $(\phi_x)_i$ is significantly greater than $(\phi_x)_o$ only during the first 30 min. This indicates an absorption ability of the roots. After 3 hrs, ϕ_x from two groups of roots are the same.

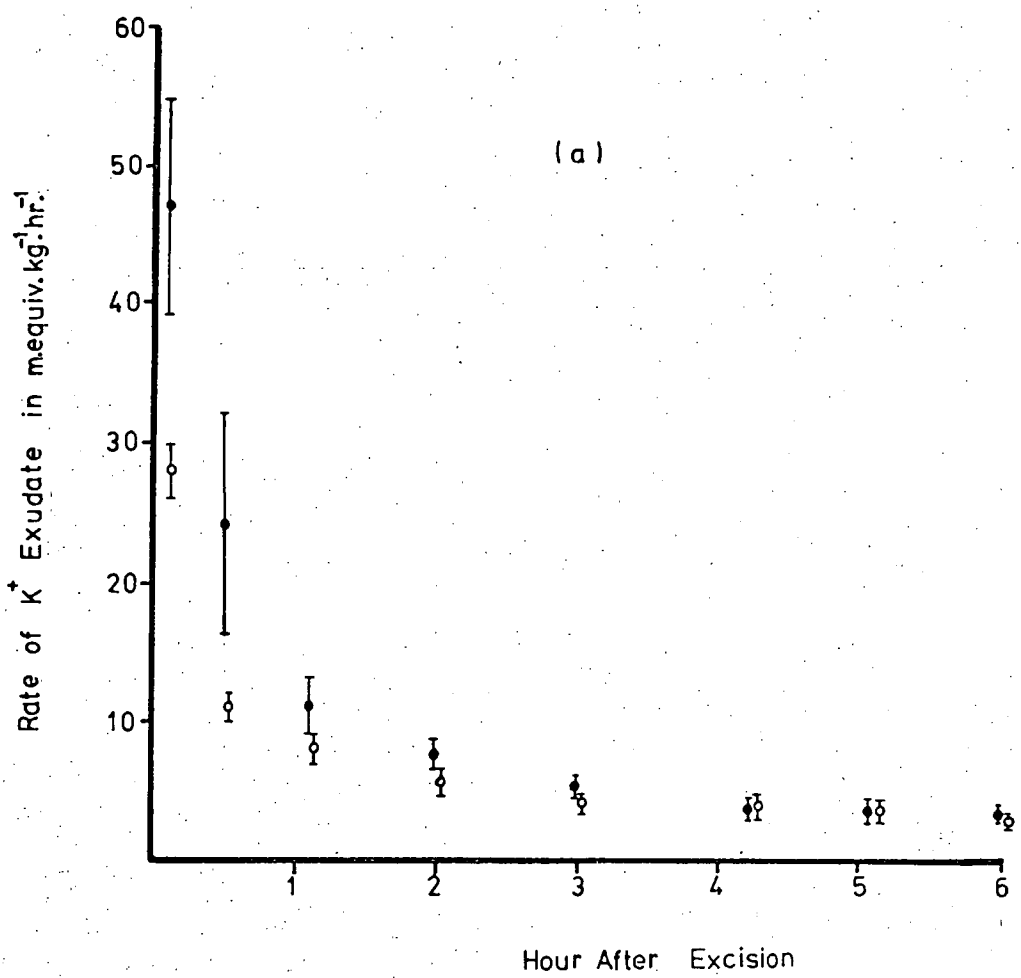
The rate ϕ_{co} of K^+ elution from this portion of roots, as shown in Fig. 5.9(b) appears in a similar fashion to that from the mature portion, but it reaches a constant value sooner (after 2 hrs) with the magnitude of 11.1 m.equiv.kg^{-1}.hr^{-1}. Taking the possible loss across the surface of the 1 mm length in the collecting chamber into account, (ϕ_x) is about 2.2 m.equiv.kg^{-1}.hr^{-1} and ϕ_v is 13.3 m.equiv.kg^{-1}.hr^{-1} (= 11.1 m.equiv.kg^{-1}.hr^{-1} + 2.2 m.equiv.kg^{-1}.hr^{-1}).

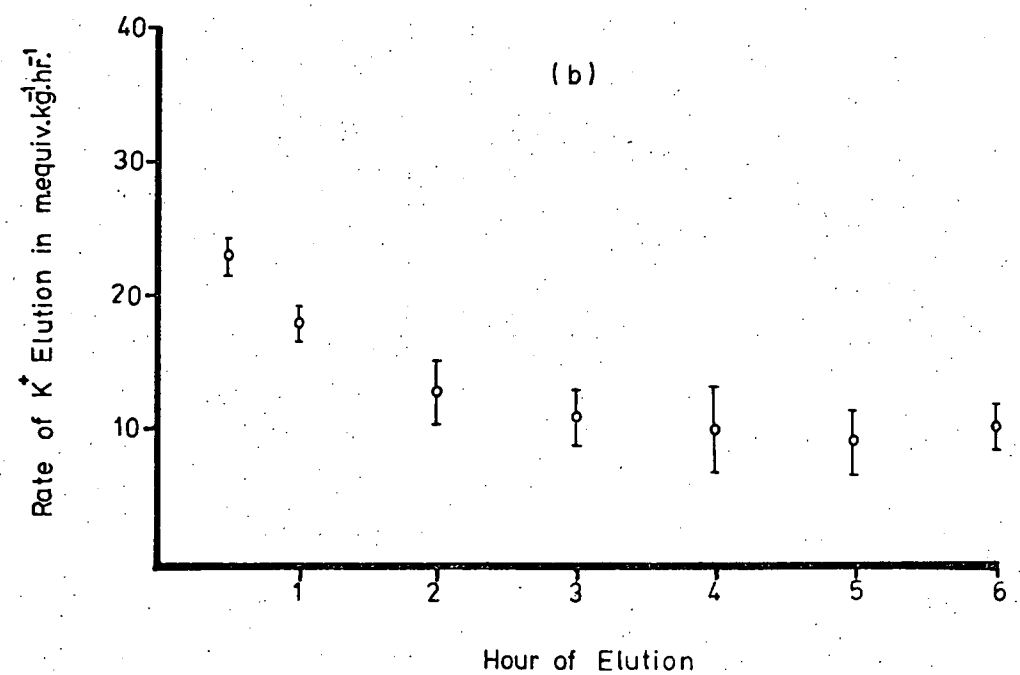
Fig. 5.9

(a) A comparison of the rate of K^+ loss through 20 mm cut end of 0-20 mm root segments when a portion between 0-10 mm from the tip was bathed in a 1x solution (●) and a K^+-free solution (○). The rest of the root was bathed in a K^+-free solution.

(b) Showing the rate of K^+ elution in $m.equiv.kg^{-1}.hr^{-1}$ from a portion between 0-10 mm from the tip of 0-20 mm root segments into a K^+-free solution.

The limit shows the standard error of the mean of 3 (a) and 2 (b) observations. Each observation contained 4 segments.


(a)
Rate of K+ Exudate in mequiv.kg-1.hr-1
60
50
40
30
20
10
1
2
3
4
5
6
Hour After Excision


(b)
Rate of K+ Elution in mequiv.kg-1.hr-1
40
30
20
10
1
2
3
4
5
6
Hour of Elution

The above calculations show that the values of ϕ_x, ϕ_v and ϕ_{co} for young cells are greater than those for mature cells, indicating a more active state of cells in excised roots than those in root segments. It should be noted that the rate of K^+ exudation at the cut end (ϕ_x) obtained in this section will be used later, in section 5.5.2, to determine the specific activity in the xylem vessels.

5.5 Data Analysis

5.5.1 Estimation of ionic fluxes and the internal contents

As it was shown in section 5.4.2 that there was a net loss of K^+ from the root tissues, the following estimations of fluxes and the internal ion contents were made utilising the analytical procedure for roots under non-steady state conditions. The equations used are from chapter 2, as follows:

$$\phi_{oc} = \phi_{in}(1 + 1/\alpha) \qquad (2\text{-}32)$$

$$\phi_{vc} = (1 + \alpha)(\phi_{in} - \phi_v) + \alpha\,\phi_{xc} \qquad (2\text{-}34)$$

$$Q_c = \frac{(\phi_{co} + \phi_{cv} + \phi_{cx})}{k_s} \qquad (2\text{-}19)$$

$$Q = \frac{\phi_{vc}}{k_L}\left(1 - \frac{\phi_{in}}{\phi_{oc}}\right) + \frac{\phi_{oc} + \phi_{xc}}{k_s} \qquad (2\text{-}21)$$

and $$s_c \simeq \frac{s_o\phi_{co}}{\phi_{co} + \phi_{cv} + \phi_{cx}} \qquad (2\text{-}41)$$

where $\phi_{co} = \phi_{oc} - \phi_v - \phi_x$, $\alpha = \phi_{in}/(k_s Y_s)$, and ϕ_{in} is obtained from Y_L/T

ϕ_v used in these equations is obtained from the rate of fall of Q between 5 hrs and about 40 hrs of the ageing (Fig. 5.4). Despite the large variation in the observations of K^+ content as a function of time, it is clear that there is a net loss from the tissues which is greater in the early stage of washout than during the period 5-40 hrs which is used in this study. The value of -1.00 m.equiv.kg^{-1}.hr^{-1} was chosen for ϕ_v for both excised root tips and mature root segments. The validity of this choice will be discussed later.

To estimate ϕ_{co}, a further value required is ϕ_x, the net flux into the xylem. This can be estimated from dY_x/dt, the rate of tracer loss through cut ends of roots (section 5.4.3). As shown in chapter 4, dY_x/dt is related to fluxes into the xylem as

$$dY_x/dt = (s_c\phi_{cx} - s_x\phi_{xc}) + s_o\phi_{ox} \qquad (4\text{-}4)$$

It was previously shown that $s_x\phi_{xc}$ and $s_o\phi_{ox}$ are small compared to $s_c\phi_{cx}$ for mature roots (section 4.4.8 and 4.4.5 in chapter 4) and it is likely that they are also negligible in excised tissue. The approximate value of ϕ_{cx} is, therefore, the ratio of dY_x/dt to s_c. By guessing a value for s_c and utilising the calculated value of ϕ_{cx}, ϕ_{co} and s_c from equation are obtained. If the guessed value of s_c and the calculated one did not agree, the process was repeated by an iterative procedure similar to that applied in section 4.5.1.

Estimated values for fluxes and the internal K^+ contents, and also other values used in the calculations, are shown in Table 5.2 for excised root tips when ^{86}Rb was used as a tracer and Table 5.3(a) and (b) for root segments when ^{86}Rb and ^{42}K were used, respectively. The values of ϕ_v and dY_x/dt used in these calculations are also shown in the tables. In some early experiments the loading period was 2 hours, but these were discontinued when it was found that s_c had not reached a quasi-steady state and dY_x/dt had also not reached its steady value. In the experiments reported in Table 5.3, the loading time was 5 hrs.

Table 5.2

Estimations of fluxes (ϕ_{oc}, ϕ_{co}, ϕ_{cv} and ϕ_{cx}) and the internal K^+(^{86}Rb) contents (Q_c and Q) in excised root tips (i.e. 0-10 mm from the tip), utilising data from Table 5.1(a) of T=5 hrs. Data used in the extimations were ϕ_v = -1.00 m.equiv.kg^{-1}.hr^{-1} (Fig. 5.4) and dY_x/dt = 5.78×10^9 cph.kg^{-1}.hr^{-1} (Fig. 5.5b).

Note that fluxes are in m.equiv.kg^{-1}.hr^{-1} and the contents in m.equiv.kg^{-1}.

	(a)				(b)		
Exp. no	ϕ_{oc}	ϕ_{cv}	Q_c	Q	s_c/s_o	ϕ_{cx}	ϕ_{co}
1	1.32	1.93	4.83	65.5	.31	.25	2.07
2	1.58	.88	3.20	94.8	.46	.17	2.41
3	1.09	4.43	12.3	106.0	.17	.47	1.62
4	1.09	1.70	7.29	162.0	.29	.27	1.82
5	.77	3.25	10.9	143.0	.15	.53	1.24
6	.70	2.69	8.61	125.0	.16	.50	1.20
$\bar{X}$ $\pm$ S.E.	1.09 $\pm$ .13	2.48 $\pm$ .51	7.85 $\pm$ 1.42	116.1 $\pm$ 14.2	.26 $\pm$ .05	.36 $\pm$ .06	1.73 $\pm$ .19

Table 5.3

Estimations of compartmental fluxes, the xylem influx and the internal contents of K^+ ions in mature root segments (i.e. 10-20 mm from the tip), utilising data from Table 5.1(b and c) of T=5 hrs.

Note that fluxes are in $m.equiv.kg^{-1}.hr^{-1}$ and the contents in $m.equiv.kg^{-1}$.

(a) ^{86}Rb Washout

Data used in the extimations were ϕ_v = -1.00 $m.equiv.kg^{-1}.hr^{-1}$ and $dY_x/dt = 3.10 \times 10^9$ $cph.kg^{-1}.hr^{-1}$.

Exp. no	ϕ_{oc}	ϕ_{cv}	Q_c	Q	s_c/s_o	ϕ_{cx}	ϕ_{co}
1	.49	1.70	5.69	114.0	.15	.28	1.21
2	.29	1.60	4.25	88.3	.10	.42	.87
3	.29	1.85	3.45	82.7	.09	.47	.82
4	.96	2.40	6.14	84.5	.22	.19	1.77
5	1.15	1.29	6.87	65.0	.33	.13	2.02
6	1.29	.58	2.13	64.0	.45	.09	2.20
7	.51	1.45	2.64	57.8	.17	.25	1.26
8	.50	1.00	2.17	57.3	.20	.21	1.29
9	.67	1.72	2.96	69.7	.20	.21	1.46
10	1.10	.53	1.67	71.6	.42	.10	2.00
11	.70	.88	2.49	51.9	.27	.16	1.54
12	.33	1.10	2.28	34.2	.14	.30	1.03
13	.24	1.74	2.98	30.2	.08	.53	.71
14	.24	1.24	2.28	56.5	.10	.42	.82
15	.20	1.16	2.48	53.6	.09	.47	.73
16	.41	.66	2.03	34.2	.20	.21	1.20
17	.23	2.22	7.05	51.6	.07	.61	.62
18	.40	.94	2.07	77.2	.17	.25	1.15
19	.21	1.21	2.59	73.6	.09	.47	.74
20	.18	1.82	4.62	79.0	.06	.71	.47
21	.45	.42	1.09	39.8	.24	.18	1.26
22	.24	.61	1.83	54.8	.13	.33	.91
23	.43	.63	3.26	44.8	.21	.20	1.23
24	.41	.66	2.91	86.2	.20	.21	1.20
$\bar{X}$ $\pm$ S.E.	.50 $\pm$ .06	1.22 $\pm$ .11	3.27 $\pm$ .34	63.4 $\pm$ 4	.18 $\pm$ .02	.31 $\pm$ .03	1.19 $\pm$ .09

(b) ^{42}K Washout

Data used in the extimations were ϕ_v = -1.00 m.equiv.kg^{-1}.hr^{-1} and $dY_x/dt = 2.44 \times 10^9$ cph.kg^{-1}.hr^{-1}.

Exp. no	ϕ_{oc}	ϕ_{cv}	Q_c	Q	S_c/S_o	ϕ_{cx}	ϕ_{co}
1	.70	3.44	7.56	108.2	.14	.24	1.46
2	.59	2.50	6.59	94.1	.14	.24	1.35
3	.79	2.76	4.84	106.0	.17	.20	1.59
4	.64	2.89	6.57	100.0	.14	.24	1.40
5	.81	1.39	4.15	80.8	.25	.13	1.67
6	.22	1.00	4.36	58.6	.10	.33	.89
7	.40	.90	2.54	78.8	.17	.20	1.20
8	.26	1.71	4.58	85.6	.09	.37	.89
9	.25	2.19	7.17	85.2	.07	.48	.77
10	.30	.76	2.37	87.1	.14	.24	1.06
11	.32	.60	2.26	56.8	.17	.20	1.12
12	.51	.76	4.44	76.0	.22	.15	1.36
13	.50	.77	3.66	90.8	.22	.15	1.35
$\bar{X}$ $\pm$ S.E.	.48 $\pm$ .06	1.67 $\pm$ .27	4.70 $\pm$ .50	85.2 $\pm$ 4	.16 $\pm$ .01	.24 $\pm$ .03	1.24 $\pm$ .08

The results show on average that in most observations, ϕ_{cv} is greater than ϕ_{co}. It was noted that when both ϕ_{in} and α are small and if they are not much different (see Table 5.4), ϕ_{cv} which varies directly to these values is smaller than ϕ_{oc} (observation #6 and #10). Note that on average, ϕ_{co} is greater than both ϕ_{oc} and ϕ_{cx} in all cases.

Diagrams representing fluxes across each cell membrane in excised roots and root segments are shown in Fig. 5.10(a), (b) and (c). Note that Fig. (b) and (c) are those of root segments when ^{86}Rb and ^{42}K were used, respectively, while Fig.(a) is from excised roots when ^{86}Rb was used. When analyses of excised root tips and root segments (Fig. 5.10a and b) are compared, it is noted that the estimates of fluxes and the compartmental contents of $K^+(^{86}Rb)$ for excised root tips are bigger than for mature root segments, resulting in the greater symplasmic specific activity.

The larger efflux from the vacuole causes a more rapid mixing of tracer in the cytoplasm and hence a smaller k_s is observed for excised root tips than for root segments. The finding of a greater K^+ content in excised roots agrees well with the results in section 5.4.2. The net loss of ions ($\phi_{oc} - \phi_{co}$) is smaller for excised roots than for root segments and a greater amount of the loss is via the root surface than via the xylem. This is consistent with the finding by chemical assay (section 5.4.5).

When the results of using ^{42}K and ^{86}Rb as tracer for K^+ ions in root segments (Fig. b and c) are compared, fluxes across the tonoplast for ^{42}K are significantly greater than those for ^{86}Rb, confirmimg that discrimination against Rb^+ is at the tonoplast not the plasmalemma. Similar to intact roots, neither ion transport into the xylem nor the efflux for $K^+(^{86}Rb)$ differ from those for $K^+(^{42}K)$. This, probably, explains the similarity for s_c/s_o between both tracers.

It should be noted that a change in dY_x/dt does not greatly affect the value of s_c since ϕ_{oc} and ϕ_{cv} are not affected. Also, the second term of the equation (2-21) can be neglected, since k_s is much greater than k_L (see Table 5.1).

Table 5.4

Showing average values of ϕ_{in} (= Y_L/T) and α (= $\phi_{in}/k_e Y_e$) obtained from washout experiments of excised root tissues.

ϕ_{in} is in m.equiv.$kg^{-1}.hr^{-1}$.

Root tissue	Isotope	ϕ_{in}	α
0-10 mm	^{86}Rb	.53±.05	1.25±.28
10-20 mm	^{86}Rb	.12±.01	.87±.13
10-20 mm	^{42}K	.24±.04	1.10±.16

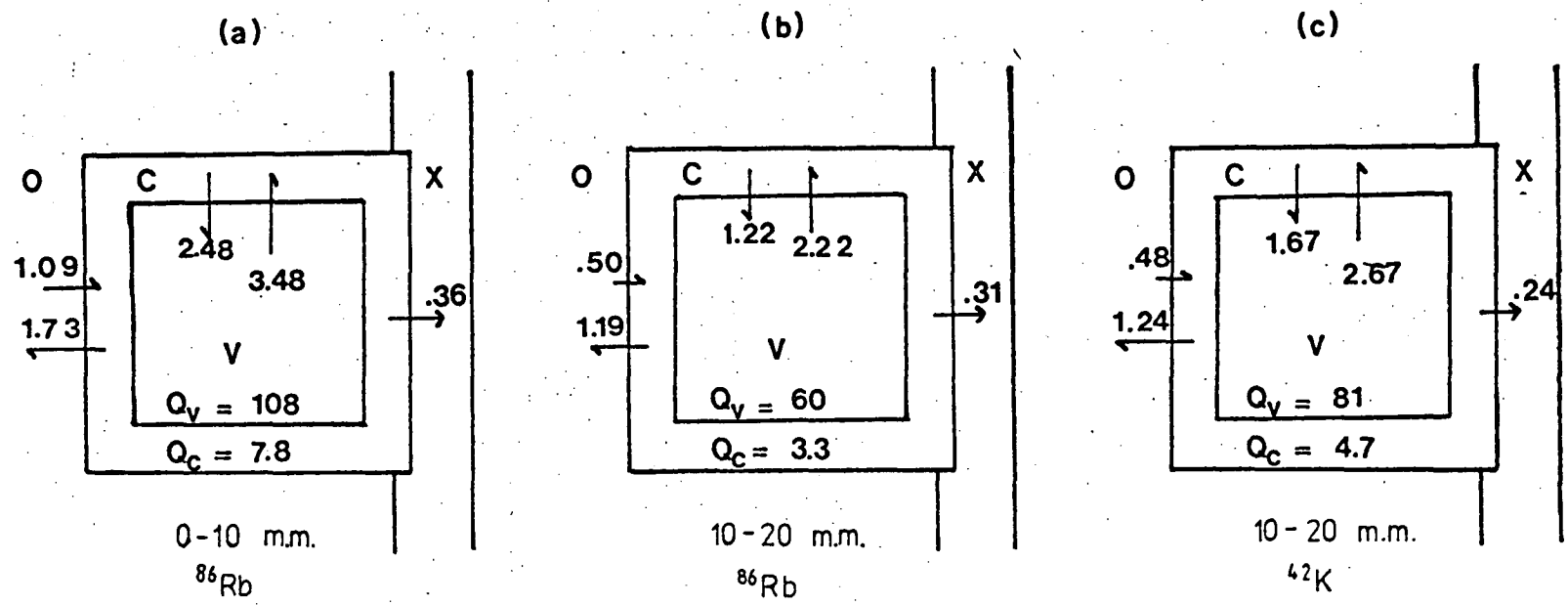


Fig. 5.10

Diagrams comparing fluxes and the compartmental contents of K^+ in excised roots (a) and root segments (b) when ^{86}Rb was used as a tracer for K^+ ions.

Fig. (c) shows those in root segments when ^{42}K was used as a tracer.

When the value of Q estimated in a flux study was compared with the value obtained from chemical assay (section 5.4.2) only the value estimated from ^{42}K studies of root segments agrees well with the assayed Q. That estimated from ^{86}Rb studies is about 27% smaller. The result confirms that ^{86}Rb is not a suitable tracer for K^+ as far as transport across the tonoplast is concerned.

5.5.2 The specific activity in the xylem

As shown in the equation (2-41) chapter 2, the specific activity of the xylem sap (s_x) is determined by

$$s_x = \frac{dY_x/dt}{dQ_x/dt} \qquad (5\text{-}3)$$

Fig. 5.5(b) shows that the rate of dY_x/dt for excised roots was 5.8×10^9 cph.kg^{-1}.hr^{-1}, while that for root segments was 6.6×10^9 cph.kg^{-1}.hr^{-1}. By direct measurement of K^+ exudation (section 5.4.5.1-2), the corresponding rates of dQ_x/dt were 2.2 m.equiv.kg^{-1}.hr^{-1} and 1.3 m.equiv.kg^{-1}.hr^{-1}, respectively.

Utilising these values, the calculated values of s_x are $0.04s_o$ and $0.07s_o$ for excised roots and root segments, respectively. It should be noted that s_x for excised roots is increasing with time due to the increasing dY_x/dt. The smaller value for excised roots than root segments, during the 6 hr experiment, is due to the lag phase of the transport.

5.6 Discussion

5.6.1 K^+ fluxes in root tissues

This study has shown that the K^+ content in excised root tissues falls with time. Analytical procedure for roots under non-steady state conditions is, therefore, utilised to estimate compartmental fluxes and ion contents and this estimation is made once the rate of fall has stabilized.

These estimates have shown that ion fluxes in excised roots are larger than those in root segments, indicating that ion movements in less mature cells are greater than in fully developed cells. This is not surprising, since the function of the root tip is to take up nutrients from the medium and use them for growth in other part of the plants.

Table 5.5 summarizes the values of K^+ fluxes in roots of several plant species grown in solutions with and without K^+ ions. These are referred to as high salt and low salt roots, respectively. Work in low salt roots shows that ϕ_{oc} is much greater than ϕ_{co}. This is also true in high salt roots of corn, but to a lesser extent. In high salt roots of corn and rice, ϕ_{cx} is smaller than ϕ_{oc} and ϕ_{co}, while ϕ_{cx} in barley roots is greater than ϕ_{co}. The specific activity in the symplast of rice is much smaller than that of corn. This could be because the absorption across the plasmalemma of corn roots is greater and tissue loading in corn was made for a longer period of time.

The finding that ϕ_{oc} is an order of magnitude greater for low salt roots than for high salt roots is an indication that the low salt roots are in an abnormal state of activity. It is therefore open to question whether the methods of analysis which assume that fluxes remain constant over a period of many hours are valid in those cases.

Although rice was grown in the same culture solution, as used by other workers for corn, there appears a net loss of K^+ from both excised root tips and mature root segments of rice. In fact, there was evidence in this study (section 5.4.5.2) showing that some uptake of ions into excised root tips did occur, but only during the first 30 min after the excision

Table 5.5.

A comparison of values of K^+ fluxes across the plasmalemma (ϕ_{oc}, ϕ_{co}) and the tonoplast (ϕ_{cv}, ϕ_{vc}), the specific activities in the cytoplasm (s_c) and the xylem (s_x) between rice roots and other plant roots, in relation to the salt status of the roots and the external concentration of the ion.

(E) = excised root, (R) = root segment, (WE) = whole excised root, (HS) = high salt root, (LS) = low salt root, (S) = steady state conditions and (NS) = non-steady state conditions

Plant	External K^+ (mM)	ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}	ϕ_{cx} (m.equiv.kg^{-1}.hr^{-1})	s_c/s_o	s_x/s_o	Note
broad bean	1.0								
(HS),(R),(S)		.46	.46	7.6	7.6	-	-	-	a
(HS),(R),(NS)		.21	.33	3.65	3.77	-	-	-	
corn	1.0								
(HS),(E),(S)		2.5	1.5	1.7	1.1	.93	.62	.39	b
barley	0.2								
(LS),(E),(S)		14.6	2.3	-	-	12.3	-	-	c
(LS),(WE),(S)		16.2	3.9	2.0	2.0	12.3	-	.94	
(LS),(R),(S)		19.5	5.2	6.5	6.5	14.3	-	.92	
rice	1.0								
(HS),(R),(NS)		.48	1.2	1.7	2.7	.24	.16	.07	d
(HS),(E),(NS)	*	1.1	1.7	2.5	3.5	.36	.26	-	

Note a - Pallaghy and Scott (1969), b - Davis and Higinbotham (1976), c - Behl and Jeschke (1982), d - the present study and * - experiments using ^{86}Rb as a tracer.

and there was no evidence of the net uptake of K^+ into root segments. This study indicates that rice roots lose the ability to absorb nutrients from the medium after being excised and the activity in root segments falls more rapidly than in roots which retain the root tip.

To test whether this loss of K^+ was related to the depletion of energy resources, a few experiments were performed in which sucrose was added to the medium. The results of these experiments were inconclusive and they were discontinued because of the difficulty of avoiding bacterial contamination.

The result from rice root segments is more compatible with that obtained from cortices of broad bean roots, since both are concerned with the movements of ions across mature cells. If the analytical procedure for roots under non-steady state conditions is used, there also appears a net loss of ions from broad bean roots.

Due to the finding of the inconsistency between the estimated Q and the chemical assayed value in ^{86}Rb studies, it is concluded that ^{86}Rb is not a suitable tracer for K^+ ions on the study of transport across the tonoplast.

It is interesting to point out that the linear part of the efflux graph which determines the rate of long-term exchange in barley roots lasted for atmost 10 hrs (Behl and Jeschke 1982). If low salt roots contain more sugar than high salt roots (Pitman 1976), it is expected that the rapid fall of ions from rice root tissues should appear sooner. However, this was not the case for rice roots. During the rapid fall, it was found in a preliminary experiment that the amount of ion loss through the cut end was also increased.

It was observed that ϕ_{co} obtained from chemical analysis (section 5.4.5) was much bigger than the value obtained by washout analysis. The cause of the inconsistency could be determined by the difference in the composition of the root medium. In the former case, the absence of K^+ ions from the medium and their replacement by Rb^+ may result in a bigger K^+ loss due to a greater concentration gradient between the cell interior and the exterior (Anderson et al. 1970). Alternatively, Rb^+ may not be a suitable ion to substitute for K^+ at 1 mM

cocentration. To test this, an experiment similar to that reported in section 5.4.1 was carried out by substituting choline chloride for RbCl in the K^+-free solution. From a single experiment, it was observed that K^+ loss from root tissue was smaller than before, though still big in magnitude compared to the value obtained from flux analysis. Accordingly, this suggests that the replacement of RbCl for KCl can cause an enhancement of K^+ loss from the tissue. The mechanism by which this enhancement takes place remains to be investigated.

In conclusion, this study reflects similaities between excised roots and root segments, except the estimated fluxes across each cell membrane of the former are larger. The fact that young cells occupy less than half of the root length in this tissue suggests that if the study was made in 0-5 mm tip region, the magnitude would have increased by at least 50%. It should be noted that the ratio of ϕ_{oc}/ϕ_{co} and ϕ_{cv}/ϕ_{vc} are larger for excised root tips than for root segments. This difference will be considered further in chapter 8.

5.6.2 The lag phase of transport into the xylem

By the use of a tracer, it was observed that an appreciable delay of transport into the xylem occurred only in excised roots (section 5.4.3). The presence of a root tip has no effect on the transport if tissue loading was made at a mature cell region. The non-lag phase in that region is in contrast to the finding in similar cells for intact roots. This could be due to the much smaller k_s for root segments.

A lag phase of transport in excised roots has been reported by several authors (Pitman 1971, Smith 1970 and Bange 1977). It is worth mentioning that in all these cases roots were in a low salt condition. These observations together with the evidence found in the present study suggest that the lag phase is independent of the salt status of the roots.

The results of ion fluxes in this chapter and in the previous one will be used in chapter 8 for a further consideration on the mechanism of K^+ transport, by utilising the Ussing-Teorell equation. To do this, information of membrane

potentials is essential. This is investigated in next chapter.

Chapter 6

Transmembrane Potential In Rice Roots

6.1 Introduction

In order to predict the flux ratio of ion transport across root cell membranes using the Ussing-Teorell equation (see chapter 8), information is required on the electrical potential of cortical cells and the xylem vessels.

As mentioned earlier, there is some difficulty in the measuring transmembrane potentials in roots of higher plants. When advancing a microelectrode into root cells, a blockage of cell debris can occur at the electrode tip and a possible short-circuitary along the ruptured cell membrane (see Anderson and Higinbotham 1975) can lead to uncertainty in the measured potentials. With this in mind, the measurements on cortical cells will be performed by advancing microelectrodes into at most three cortical cells.

It is known that rice can adapt well to oxygen deficiency. John et al. (1974) demonstrated that the abilities of adapted plants to take in nutrient from the medium is equal to that of aerobically grown plants in terms of ion uptake. It is of interest to investigate electrical cell potentials of plants grown in both conditions. In the accepted manner of many authors, oxygen deficient condition was met by passing nitrogen gas (Cheeseman and Hanson 1979b, John et al. 1974 and Mocquot et al. 1981) or carbonmonoxide (Anderson et al. 1974), instead of air, into the root medium.

In this chapter the measurements were also made with an energy inhibitor so that an electrogenic ion pump potential could be estimated. Inhibitors which are commonly used are 2,4 Dinitrophenol (DNP), Cyanine (CN^-) and sometimes Azide (N_3^-) (Davis and Higinbotham 1969, Higinbotham et al. 1970, Anderson et al. 1977 and Drake 1979) . However, since CN^- was thought to induce CN^--resistant respiration in plant tissues (see reviews by Laties 1982, and Solomos 1977), DNP was chosen for this study.

It is commonly found that cortical electropotential is negative relative to the external medium. In

freshly excised roots, the magnitude of the potential is small, becoming significantly more negative with ageing. This seems to be the case for both low salt roots whose excised root tissues were aged in solutions of varied concentrations (barley-Pitman et al. 1970) and for high salt roots aged in a culture solution (sunflower-Graham and Bowling 1977). The cause of these potential changes is not fully understood. In contrast to the above, Mertz and Higinbotham (1976) investigated the effect of ageing on electrical cell potentials of high salt corn roots and did not find a significant difference in cortical cell potentials between intact roots and freshly excised roots, provided the measurements in excised roots were made about 1 mm away from the cut surface. They suggested that the change in the electropotentials of cells at a cut surface after ageing was due to a physiological change in the tissue.

The use of different culture solutions by the above workers makes it difficult to judge whether the change in the potentials after excision is due to the movement of a particular ion species or to the nature of the plant root itself. Investigation of the ageing effect on cortical cell potentials of high salt roots of rice was made by using a culture solution as used by Mertz and Higinbotham (1976) and the results are discussed in comparison to that of other plant species.

6.2 Experimental Materials

6.2.1 Preparation of A- and N-roots

Seedlings were grown as described in chapter 3, section 3.3.1. Plants used in this experiment were between 4-6 days old, unless otherwise stated. Since continuous aeration was provided to the root medium throughout the plant's life, the roots of these seedlings were referred to as A-roots. In some experiments (section 6.4.2), N-roots were used. They were prepared by passing N_2 gas through the root medium for 5-6 hrs prior to the experiment. All seedlings were left under the measurement conditions for about 5 hrs before the experiment commenced, except for those in which the immediate effect of a

change in medium was to be investigated.

6.2.2 Root bathing medium

The root bathing medium used throughout this experiment is a 1x solution as described in section 3.2. To study the effect on membrane potentials of a change in the oxygen state of the root medium (section 6.4.2), separate oxygenated and nitrogenated solutions were used. They were prepared, respectively, by passing air and N_2 gas bubbles continuously through aspirator bottles containing a 1x solution. The bathing solution was passed to the root from the appropriate aspirator bottles through a plastic tube.

6.2.3 Microelectrodes, Half cells and Salt bridges

The microelectrodes used in this experiment were made from Borosilicate capillaries, supplied from Clark Electromedical Instruments, U.K., with a fine filament fused to the inner surface and the outside diameter was 2.0±0.1 mm, pulled by a standard Hodgkin puller. The standard size of microelectrode tip was 1 μm or less. Since the approximate size of the electrodes could not be observed through the 300x microscope, it was determined by tip resistance (Robinson and Scott, 1965). An acceptable range of the tip resistance was 10-40 M ohms. Every electrode was freshly prepared to avoid foreign material which could cause a high tip resistance. The inner filament of the electrode served as a wick to conduct an electrolyte, 3 M KCl solution, down to the tip of the electrodes when filling.

Ag-AgCl half cells were prepared by coating Ag-wires (0.125 mm in diameter) with Cl^- ions in a weak HCl acid solution (0.1 N concentration) for 5 minutes. One half cell was used to connect the microelectrode to an electrometer (see Fig. 6.1) and another was used as a reference electrode by connecting it between a salt bridge and an electrometer. The salt bridge was a plastic tube filled with 3 M KCl plus 2% agar. They were prepared in bulk and stored in a 3 M KCl solution until used.

6.3 Experimental Methods

A microelectrode was connected to an Ag-AgCl half cell whose other end was connected to the amplifier input of a micro-probe system, W-P Instrument model M701 (see plate 6.1). The input and the output impedance of the system were 2×10^{10} ohms and 800 ohms, respectively. The tip resistance test facility was provided in the system and allowed a calibrated current of 1.0 nanoamperes to pass through the microelectrode. This produced a dc shift of 1 mV per M ohm at unit gain of the amplifier. The measurement was recorded on a Tohshin Electron chart recorder which was directly connected to the micro-probe system (Electrometer). The measuring circuit is shown in Fig. 6.1(a). The microelectrode was advanced into the cell by means of a micromanipulator (Hodgkin-type). The insertion was viewed through a 300x magnification microscope.

When a seedling was transferred to the experimental container, only the root was allowed to be in the bathing solution. The inlet and outlet of the solution were arranged in such a way that the root under measurement was not disturbed by the flow (see Fig. 6.1b). The rate of flow was about 1.5 ml.$(min)^{-1}$. This is presumed to be enough to wash away ion content lost from the cells during electrode impalement.

As the electrode tip was inserted in the tissue, it usually became partially blocked with cellular debris and its resistance became much higher (80-150 M ohms). This high resistance remained after the tip was withdrawn from the tissue unless the tip was broken. All measurements in this chapter were performed at a controlled temperature of $24(\pm 2)^{\circ}$ C.

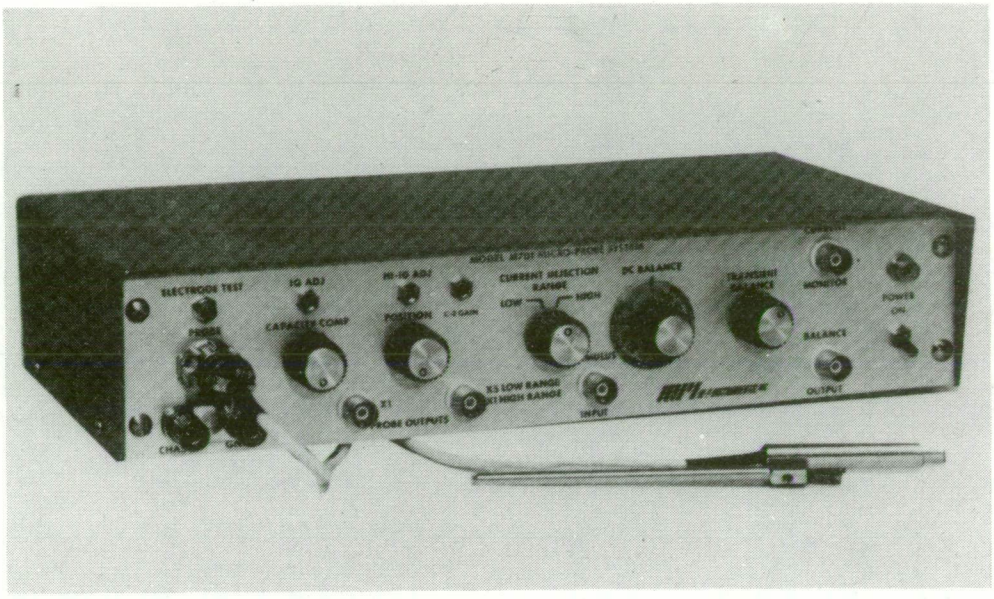
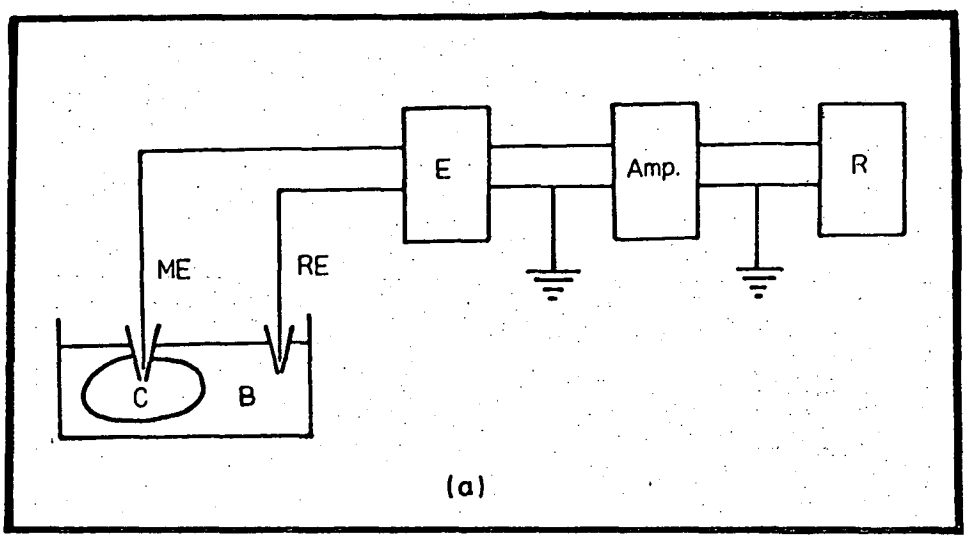


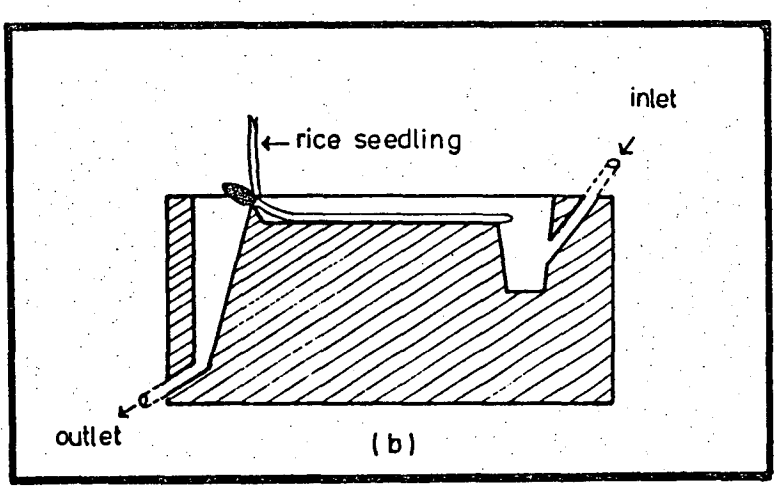
Plate 6-1.

A micro-probe system, W-P Instrument model M701, which was used in this study.

Fig. 6.1



(a) Block diagram of transmembrane potential measuring circuit. The potential was recorded on a Tohshin Electron chart recorder (R) which was connected in parallel with an electrometer (E), W-P Instrument model 701. The electrometer contained build-in amplifiers of $2X10^{15}$ ohms-input and 800 ohms-output impedance. C represent living system, B: bath container (see figure 6.2b), ME: measuring electrode and RE: reference electrode.



(b) A half diagram of root bath container which was made of a slab of perspex of 1 mm thick sandwished in 2 slabs of perspex of 30x90x1 mm each. The inlet and outlet were drilled through the middle slab. The window for viewing the root was a glass cover slip which was glued to the middle perspex.

6.4 Results

6.4.1 Membrane Potential Profile of intact roots

Potential measurements were performed at a distance of 2-25 mm from the tip. Cytoplasmic streaming was observed only in young hair cells and this allowed the cytoplasmic phase to be distinguished clearly from the vacuolar phase. The streaming was used as an indication for healthy roots. It was observed that if unhealthy roots were used, the cell wall was deformed during cell impalement, sometimes leading to a broken tip. This also happened when the root lost its internal content to some extent after several withdrawals of microelectrodes. To avoid this problem, each root was impaled not more than 3 times and at distances of at least 2 mm apart.

It was found in preliminary work (see Fig. 6.3) that the cortical cell potential was independent of the distance from the tip. Most of the measurements were, therefore, performed in younger cells at a distance of less than 10 mm from the tip where the streaming in root hair cells could be more easily observed.

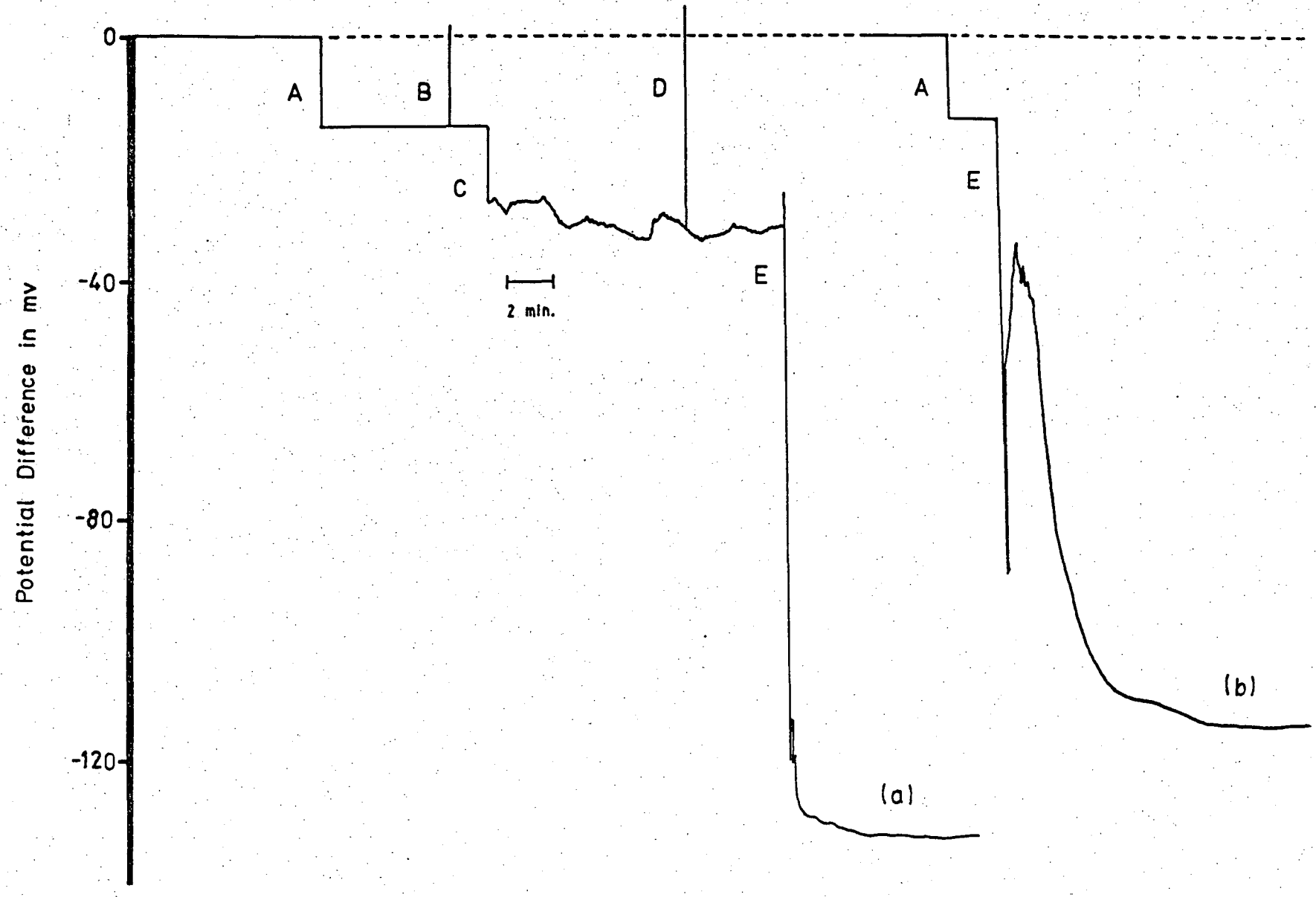
For geometrical reasons, it was difficult to observe the position of the microelectrode tip as it was advancing into the root epidermis. Consequently, the position of the tip in the cell had to be inferred from electrical measurements. Following the report by Dunlop (1976), it was considered to be in a cell if the potential changed abruptly, as shown in Fig. 6.2(a). However, on some occasions, the measurement showed a rapid depolarization followed by a slow hyperpolarization (see Fig. 6.2b). Such depolarization was, presumably, caused by some blockage of the electrode tip, since it was found that the tip resistance was large (i.e. between 80-120 M ohms) during the depolarization and became gradually smaller when hyperpolarization was taking place.

Since the cytoplasm thickness of higher plant cells is in general less than 1μm while the vacuole occupies about 85-90% of the cell volume (Higinbotham 1970), the position of the tip was most probably in the vacuole. However, an attempt

Fig. 6.2

Showing two patterns of cell potentials after advancing a microelectrode into root cells; (a) an abrupt change of the PD with a steady value thereafter and (b) a rapid depolarization before the steady value was acheived.

A represents the potential recoded when both electrodes were in root bathing medium (1x solution), C: when the measuring electrode was pressed against root cell wall and E: when it was in an epidermal cell. The tip resistence was changed from 20 M ohm in the root medium (B) to 40 M ohm in the cell wall(D).


0
-40
-80
-120
Potential Difference in mv
A
B
C
D
E
2 min.
(a)
A
E
(b)

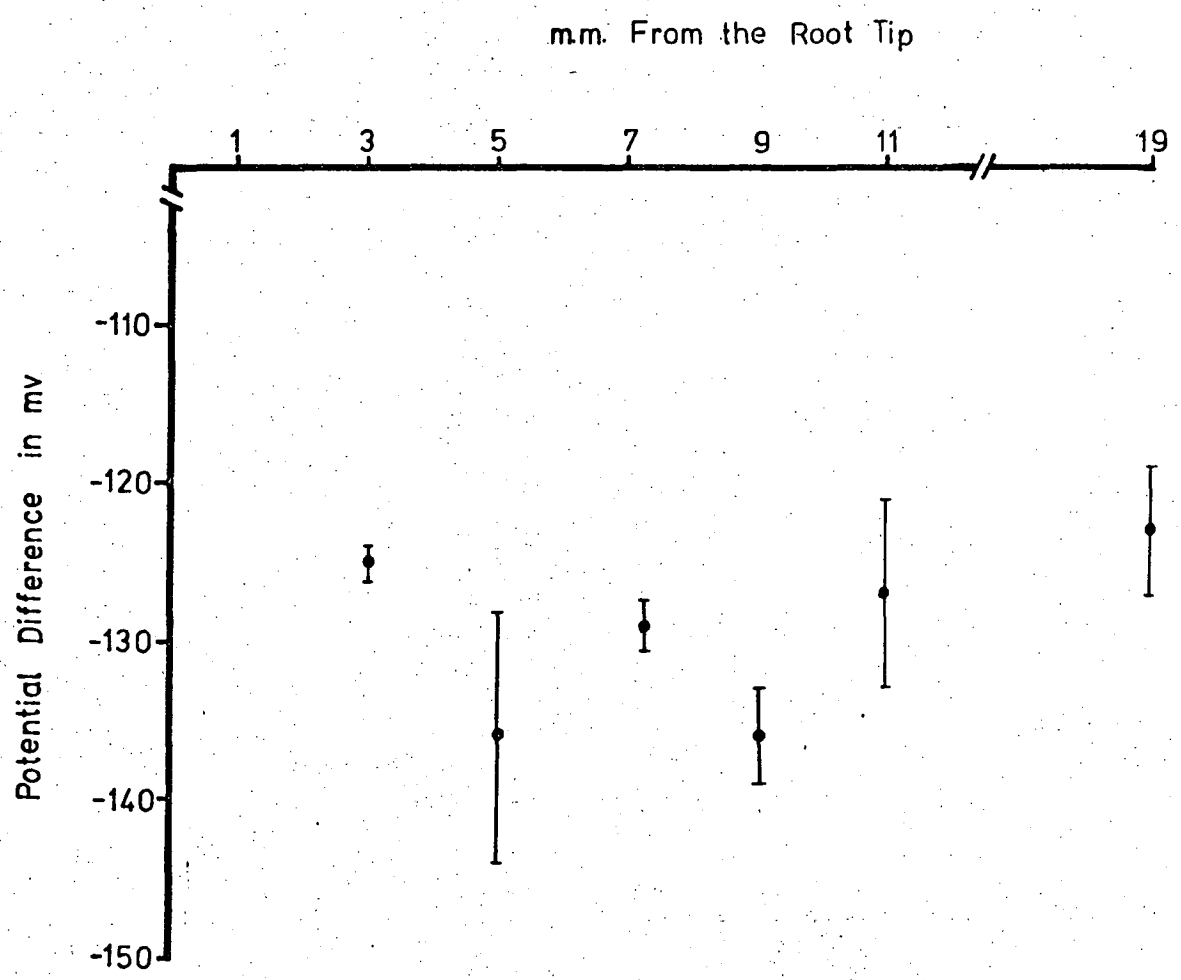


Fig. 6.3

Showing cortical cell potentials measuring at different position along intact A-roots of 5 days old seedlings. The limit is the standard error of the mean of 4 observations (4 roots) for each data point.

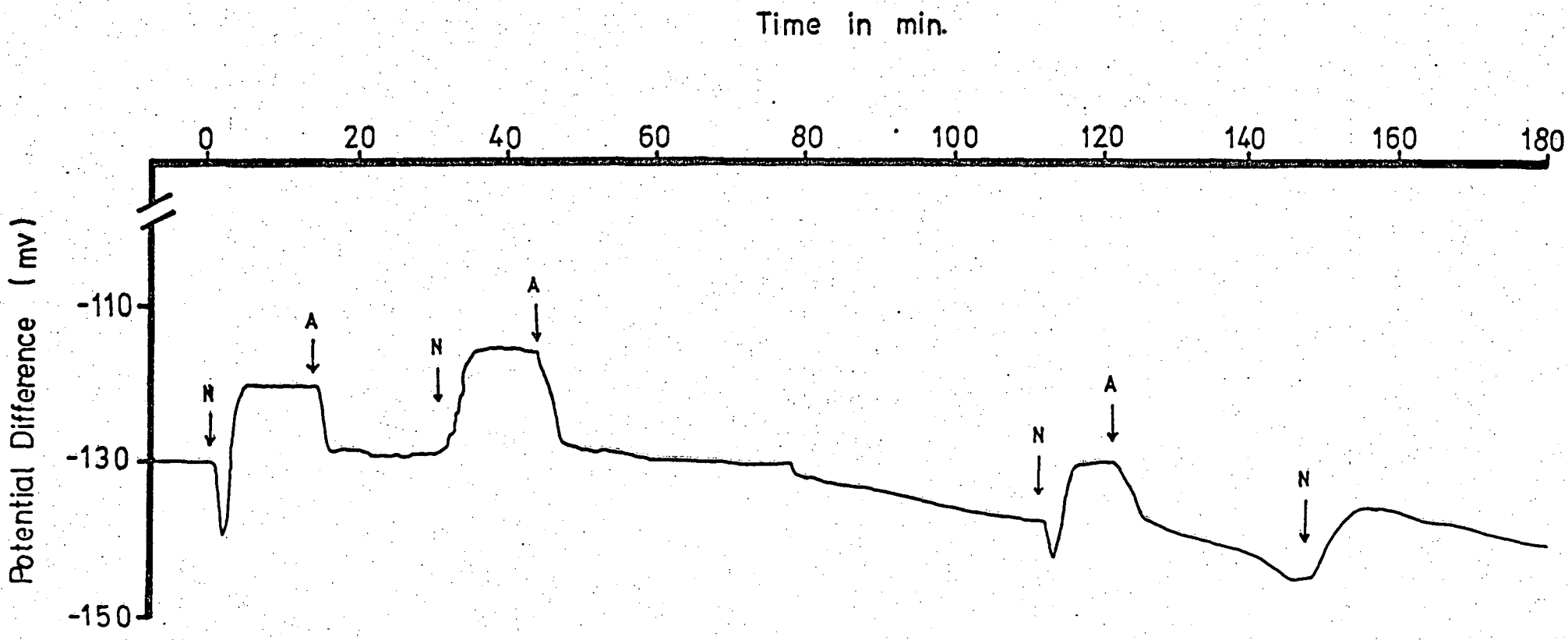


Fig. 6.4

Changes in cortical cell potentials of an A-root when the bath solution was changed from aerated (A) to nitrogenated solution (N). The external medium was a 1x solution which was a weak phosphate buffer. The transient hyperpolarization occurred when the root was first subjected to the nitrogenated solution and after it was recovery in the aerated solution for a period of time.

to measure the potential across the tonoplast was made by inserting microelectrodes into root hair cells. In a series of 8 measurements, there was no observable potential change while the electrode was advanced into the vacuole. On this evidence, the potential across the tonoplast is presumably negligible.

For each cell impalement, the reading was taken after the potential had stabilised for about 5 minutes. A further advance of the electrode to the next cell was then performed. The cell wall potentials were recorded when an electrode was pressed against the wall (see Fig. 6.2a). On the impalement of the hair cells, cytoplasmic streaming ceased for about 3-5 min before the circulation re-started.

Table 6.1 shows a potential profile from the epidermis to the third cortical cells. The measurements from hair cells are also shown. There is no significant potential gradient in the radial direction across the outer cortex. On average, the cortical cell potential is -132 ± 2 mV, ranging from -110 mV to -160 mV. The cell wall potential is -48 ± 4 mV which is small compared to cortical cell ones.

6.4.2 The effect of changes in the oxygen state of the root medium on cortical cell potentials

In order to investigate how the potential of the cortical cells responded to the change in the oxygen state of the root medium, oxygenated and nitrogenated solutions described in section 6.2.2 were used. These were prepared about 3 hrs prior to the experiment and bubbling continued throughout the measurements. The experiments were carried out using A-roots (see section 6.2.1).

After the cortical cell PD of an A-root had remained steady for about 5 minutes, the root medium was changed to the nitrogenated solution. Fig. 6.4 shows the change in the PD after the roots were subjected to the solution. The PD reverted to the original value when the solution was replaced with the aerated one. From an average of 10 measurements in 10 roots it was found that cortical cells depolarized by 11 ± 2 mV in the nitrogenated solution.

Table 6.1

Electrical membrane potential profile of rice root cells from the cell wall to the outer layers of cortical cells. The parenthesis indicates number of experiments/number of roots. The limit is standard error of the mean.

Cell wall (mV)	Root hairs (mV)	Epidermal (mV)	Cortical cells (mV)		
			1st	2nd	3rd
-48 ± 4 (12/10)	-132 ± 2 (8/4)	-129 ± 4 (18/14)	-133 ± 3 (19/10)	-127 ± 3 (15/10)	-133 ± 3 (14/9)

It is interesting to note that before the depolarization occurred, there was a transient hyperpolarization which took place within one minute. The magnitude of this transient change was 9±1 mV, more negatively. Although the solution itself was a weak phosphate buffer (1x solution), it might be argued that the transient change could be due to an induced-pH change of root cells when being subjected to the nitrogenated solution. A further experiment was carried out by using a MES buffer solution. This was prepared in bulk at 100 mM concentration with an addition of NaOH to adjust its pH to 5.5. An appropriate quantity of this solution was added to the 1x solution. This buffered solution was prepared in bulk and equal amounts transferred to two aspirator bottles; one for aeration and the other for nitrogenation. The results were similar to those obtained from the weak phosphate buffer solution. Fig. 6.5 compares the results obtained from both solutions. As shown, in the two cases in which nitrogenated solution was introduced to the root medium, the cells depolarized by about 10 mV.

It was often found that the transient hyperpolarization disappeared for the second or the third change of the root medium to the nitrogenated solution, unless the root was left in an oxygenated solution for a period of time (Fig. 6.4). In later experiments, it was found that a period of 30 minutes recovery in an aerated solution was long enough for the transient change to re-appear.

Fig. 6.4 also shows that if the bathing solution was replaced alternatively by the oxygenated and nitrogenated ones, a trend of hyperpolarization toward the original PD appeared. Since this occurs slowly, the potential change is likely to be due to some adjustment of the root to the new environment rather than any fault occurring at the electrode tip. However, the PD within the same cell was rarely recorded successfully for a long period of time. Frequently, there was a sudden fall of cell PD which is, presumably, an indication of an inactive cell state.

It should be noted that transient hyperpolarization was also observed in a few experiments in which whole excised roots were used. This suggests that the transient

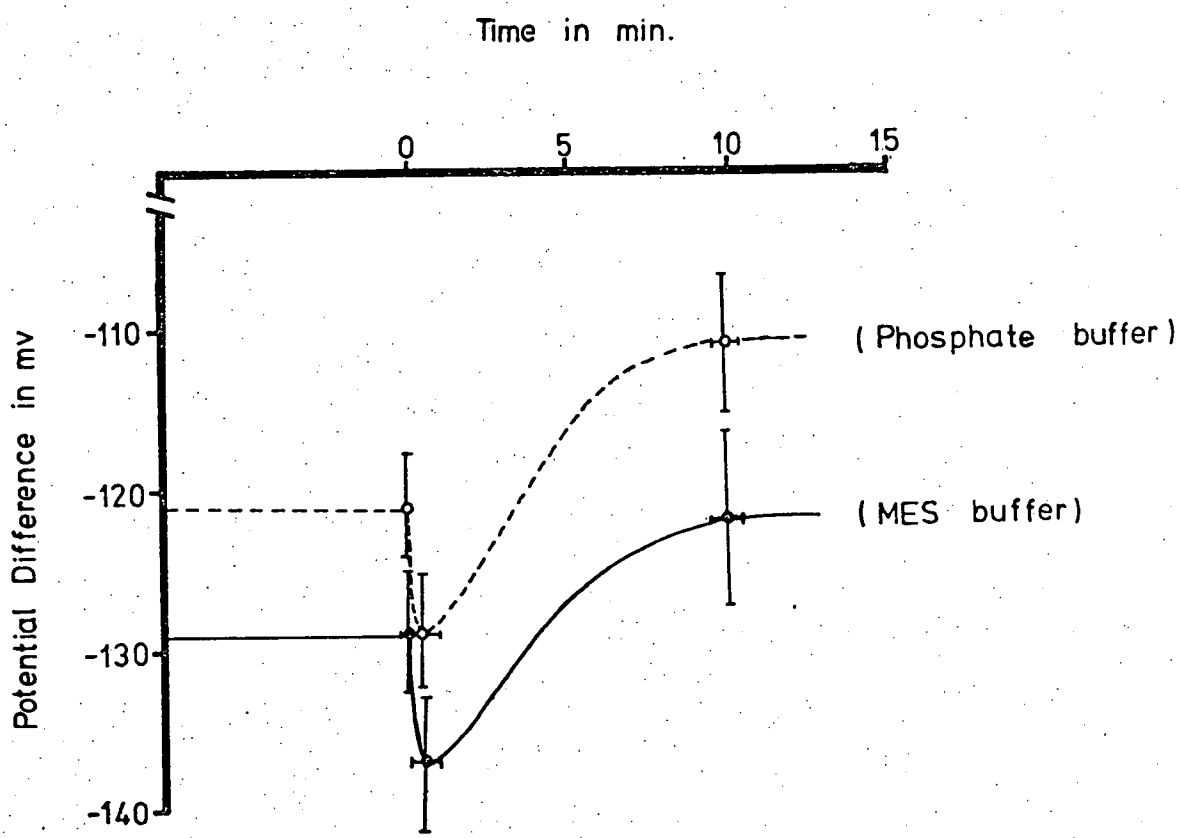


Fig. 6.5

Showing a transient hyperpolarization and depolarization of intact A-roots after root bathing solution was changed from an aerated one to a nitrogenated one. (o) shows potential changes when the medium was a weak phosphate solution (i.e. a 1x solution) and (●) in a MES buffered solution. The limit denotes the standard error of the mean from 10 measurements in 10 roots for (o) and 9 measurements in 7 roots for (●). pH value for both solutions was 5.5.

term was not controlled by the seed or shoot.

The above measurements show that the cortical cell potential measured in an oxygen deficient solution is smaller than that measured in a well aerated solution. Since rice can normally withstand an anoxic condition of the root, it is of interest to test whether a long-term pretreatment of the root in an oxygen deficient solution can bring the cell PD close to that of A-roots.

The pretreated roots which will be referred to as N-roots were prepared as described in section 6.2.1. To assure that the root environment did not change during the measurement, a bulk solution in the aspirator bottle (approximately 1 litre) was nitrogenated throughout the measurement and for about 5 hrs prior to the experiment. Cell PD of N-roots were, thus, recorded in the nitrogenated solution. These roots were only exposed to air for about half a minute during transfer from the preparation system to the measuring system. It was found that cortical cell PD varied from -100 mV to -152 mV, measured between 6-20 mm from the tip. Averaging 35 measurements in 16 roots, the potential was -122 ± 2 mV. The limits gives the standard error in the mean.

The above PD of N-roots is, therefore, about 10 mV less negative than that of A-roots. This result is consistent with that obtained from A-roots when they were measured in oxygen deficient solution. The results obtained from both groups of roots confirm that the PD of adapted root cells is smaller than aerobically grown roots. The enhancement of hyperpolarization shown in Fig. 6.4, after changing the bathing medium alternatively between oxygenated and nitrogenated solutions requires further investigation.

6.4.3 Cortical cell potentials of excised root segments

In this experiment, 7 day old seedlings were used. Roots were excised at 5 mm and 15 mm from the tip and aged in a 1x solution with continuous aeration. Since root segments lose salts with ageing (chapter 5, section 5.4.2), the solution was changed twice a day to avoid effects caused by any undue change of solution concentration. Both ends of a segment were

held by small pieces of sponge in the bath container. Cell impalement was made at a distance of 3-5 mm from either cut end.

Fig. 6.6 shows cortical cell potentials obtained from the segments after different periods of ageing. The zero hour one represents those of freshly excised segments. The cell PD of freshly excised roots was small. Within 2 hrs of ageing, root cells become hyperpolarized. By the end of about 50 hrs, the average PD obtained from 8 observations is 126±8 mV. This is close to the value obtained from intact roots (-132 mV, from section 6.4.1).

6.4.4 Electrical potential of the xylem sap

It was observed in preliminary measurements that a microelectrode after being advanced into the tissue to a certain depth was usually bent. This could be due to pressure of the tip against the tough wall of endodermal cells. Two parallel dark bands along the root were observed at the inner cell layer and this, presumably, is the layer of the endodermis. After straightening the microelectrode, the cell potential depolarized by several millivolts, as reported by Anderson and Higinbotham (1975). Attempts to measure the xylem potential of intact roots were therefore discontinued. The measurement was made in freshly excised roots instead.

Roots between 4 and 6 days old were harvested and blotted with filter paper. A water repellent grease was used to seal around the root at a distance of 5-6 mm from the tip and above the bathing solution level. This was to prevent movement of solution along the root which can cause a short circuit between the exterior and interior of the root. The root was, then, transferred to a bath containing aerated 1x solution. Two pieces of sponge were used to hold the root which was, then, excised at a distance of a few millimeters above the grease.

It was observed under a microscope that the sap oozed out from the stele and formed a hemispherical drop at the cut surface. This droplet is presumed to be the exudate from the xylem vessels. A microelectrode was lowered to touch the droplet. It was observed that the potential was slightly greater at first

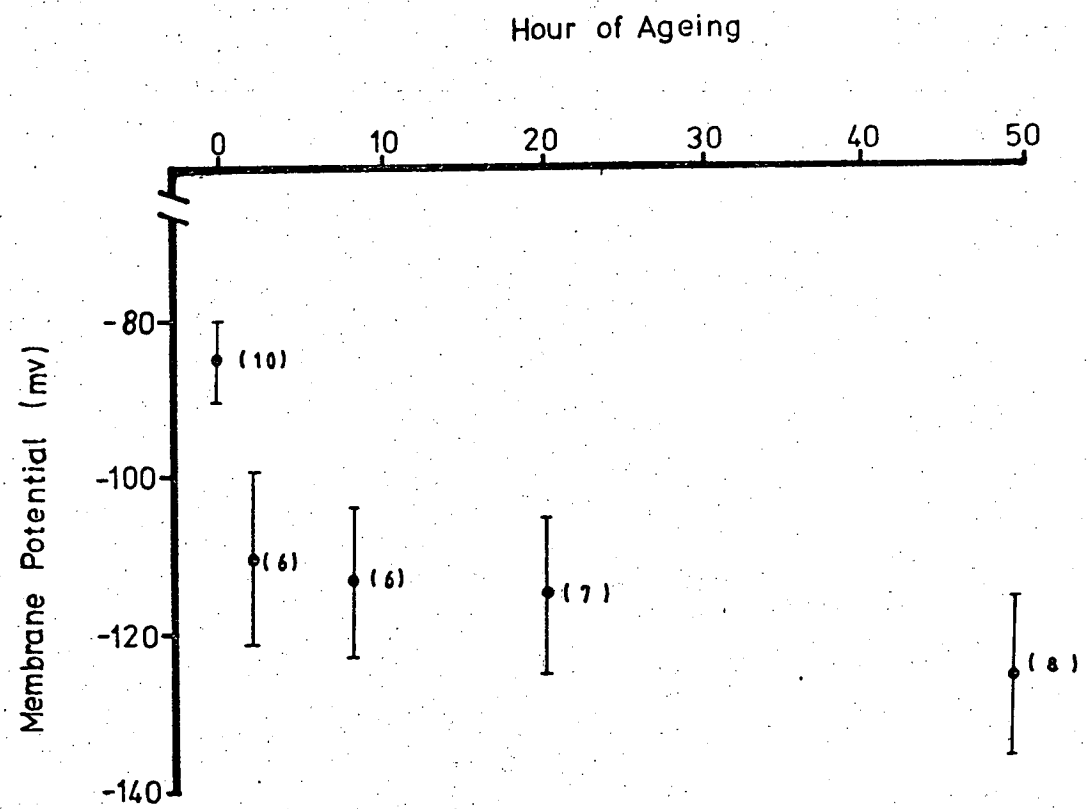


Fig. 6.6

Showing cortical cell potentials of excised 5-15 mm segments of rice roots after ageing for periods of time in the culture solution, under the same condition as growth and with a continuous aeration. The bracket indicates number of observations. The limit is the standard error of the mean.

(-70 mV on average) then gradually decreased to a steady value, normally within 10 min. The potential relative to the solution was recorded after a steady value had been reached. The average xylem PD for 10 roots was -35±4 mV. In comparison to the cortical cell PD, the xylem is 97 mV more positive.

6.4.5 The effect of 2,4-Dinitrophenol (DNP) on cortical cell potentials

It is known that the transmembrane potential comprises both the diffusion potential and the potential produced by ion pumps (see Spanswick 1981 and Cheeseman and Hanson 1979a and b). An energy inhibitor is normally used to separate these two. The potential measured under such inhibition would be expected to be close to the diffusion potential. It is of interest to investigate the magnitude of the potential caused by ion pumps in intact rice roots. Two sets of a 1x solution were used in this experiment. One was used as a control and the other contained DNP, both were buffered with MES.

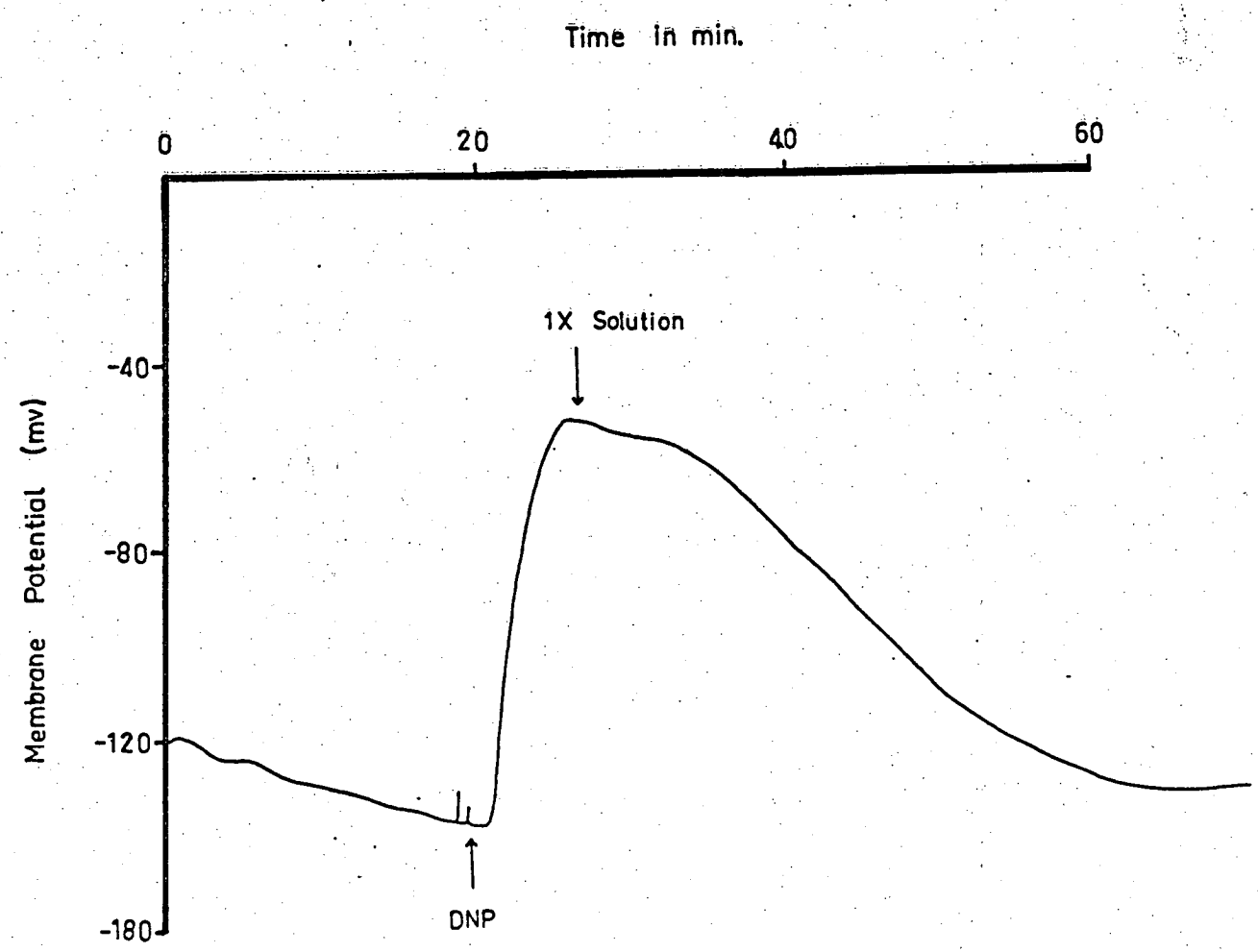
In preliminary experiments, it was found that when 10^{-3} M concentration of DNP was used, cells failed to recover after changing the solution back to normal 1x solution. In later experiments, the concentration of DNP was reduced to 10^{-4} M. The solution was introduced into the root medium after the cell PD measured under a 1x solution had reached a steady value.

Fig. 6.7 shows the cell PD before and after the DNP was introduced into the root medium. It is reversible when the DNP containing solution is withdrawn. After the withdrawal, cell recovery occurs much more slowly than the depolarization. The average magnitude of the depolarization of cortical cell potentials from 10 observations was 70±5 mV, changing from -124±5 mV to -54±3 mV. This magnitude is, therefore, accounted for by the metabolic-linked component.

It was noted that soon after the roots were exposed to DNP, cytoplasmic streaming ceased. Streaming resumed sometimes before the potential had reached the original value. The recovery time for the potential was about 45 min after the

Fig. 6.7

Showing depolarization of cortical cells from intact rice roots after the root bathing solution (1x solution) was changed to a 10^{-4} DNP containing solution. The measurement was made at about 25° C.



solution was replaced by a 1x solution. This period of time is similar to what was observed in pea epicotyl tissue after treated with CN^- containing solution (Higinbotham et al. 1970) and in sunflower roots with DNP (Graham and Bowling 1977).

If the -54 ± 3 mV potential is a diffusion potential, the result from section 6.4.2 suggests that under oxygen deficiency, cell potential still comprises both components and the metabolic-linked one is reduced by only 1/7 of the total.

The effect of DNP on the cortical cell PD of freashly excised root segments was investigated in a few experiments. It was found that PD changed from -106 mV to -50 mV. The metabolic-linked component was about 60 mV, slightly smaller than that of intact roots. Since the average PD in freshly excised roots (-85 mV) was much smaller than that of intact roots (-132 mV), this result suggests that the passive diffusion component of the cell PD is directly affected by root excision.

6.5 Discussion

Since no tonoplast potential was observed in root hair cells, it is assumed that the tonoplast potential in cortical cells is also negligible. All electrical potentials reported in this chapter are assumed to be those between the medium and cell vacuoles. This agrees with Etherton and Higinbotham (1960), Graham (1966), Nobel and Craig (1971), Lüttge and Zirke (1974) and Dunlop (1976) who considered that the potential between the vacuole and the cytoplasm was small. Table 6.2 summaries electrical cell potentials of cortical cells and the xylem in relation to the bathing medium obtained from other plant roots.

As is shown, the cortical cell potential of rice roots is similar to that of beetroots (Poole 1966), broad bean (Scott et al. 1968) and *Atriplex hastata* (Anderson et al. 1977). The potential in the xylem exudate of freshly excised rice roots was -35 mV on average. This value is consistent with that of -40 mV in corn roots, by Davis and Higinbotham (1969). Using intact roots of sunflower, Bowling (1972) advanced microelectrode from the epidermis into the stele and found the xylem potential of -27 mV, relative to the medium.

In view of possible regulation of the shoot on root cell potentials (see Graham and Bowling 1977), the PD of the xylem obtained in intact roots of sunflower (Bowling 1972) gives some confidence that the value obtained from excised roots of rice is reasonable.

6.5.1 On the potential profile of rice roots

It has been demonstrated that, under the study conditions there is no significant difference in the electropotentials between epidermal and cortical cells (section 6.4.1) of rice roots and the potential of cortical cells is independent of distance from the tip. In a region close to the root tip, the main site of ion absorption into the symplast is likely to be at the plasmalemma of both the epidermal and cortical cells. However, in the mature region where the net

Table 6.2

A summaries of cortical cell and xylem potentials of plant roots in the same culture solution as growth.

Note I = intact root
E = excised root segment
ET = excised root with the root tip.
Tr = trans-root measurement
Ex = exudation
* = plus 100 mM NaCl concentration

Root	Ext. K^+ (mV)	potential Cortical (mV)	xylem (mV)	Reference
Atriplex	1.0*	-130(I)		Anderson et al. 1977
Beet	1.0	-140(E)		Poole 1966
Broad bean	1.0	-130(I)		Scott et al. 1968
Corn	1.0		-40(Ex)	Davis and Higinbotham 1969
	1.0	-109(ET)		Davis 1972
		-177(I)		Mertz and Higinbotham 1976
		-175(E)		Mertz and Higinbotham 1976
	1.0		-32(EX)	Dunlop and Bowling 1971(b)
Ryeglass	0.4	-127(ET)		Dunlop 1976
Sunflower	7.0	-35(I)	-27(Tr)	Bowling 1972
	1.0	-150(I)		Graham and Bowling 1977
white clover	0.16		-90(Tr)	Dunlop 1982
Rice	1.0	-132(I)	-35(Ex)	The present study

absorption into root cells is small (Table 4.7), it is possible that the cortical cells lose their absorption ability to some extent, probably, due to cell differentiation (Van Iren and Van der Sluijs 1980b). On specialization of root tissues in ion transport for water plants, *Trianea bogotensis*, Vakhmistrov (1981) found the greater number of plasmodesmata which was associated with the greater K^+ activity in root hair cells than epidermal cells. Since it is generally accepted that plasmodesmata facilitates ion flow in the sympalst, it is reasonable that root hair cells were responsible in maintaining K^+ concentration gradient in the symplast, as was suggested*. K^+ activity profile, as measured by Dunlop and Bowling (1971), is possibly another interesting aspect to be investigated in rice roots before one can assure the main absorption site of ions into the symplast.

Since there was an evidence in this study (section 4.5.1) showing that most of K^+ ions which were transported into the xylem were from the vacuoles rather than from the external medium, it is suggested that ions in the symplast come from both the external medium and cell vacuoles.

Despite the variations in function of root cells from the growing region to the mature region, the membrane potential is remarkably constant. Although K^+ content in moles per 2 mm segment for the tip region (1-5 mm from the tip) was smaller than that for the mature region (15 mm from the tip) (see Fig. 3.5), the amount per unit fresh weight for the former (i.e. 150 $m.equiv.kg^{-1}.hr^{-1}$) was greater than the latter (i.e. 130 $m.equiv.kg^{-1}.hr^{-1}$). The concentration change does not seem to be big enough to alter the diffusion component of the membrane potential (i.e. the Goldman-Hodgkin-Katz equation-see Cheeseman and Hanson 1979a). However, since it is unlikely that ion species in living system move independently across cell membranes, the movements of other ions such as Cl^- or Na^+ may be involved. Besides, there may be a compensation between diffusion component and ion pump component of the potential along the root.

* by Vakhmistrov (1981)

6.5.2 The cortical cell potentials of rice roots under oxygen deficiency

As occurs in many plant tissues, such as corn roots (cheeseman and Hanson 1979b) and pea stem (Anderson et al. 1974), rice root cells depolarized after they were subjected to oxygen deficiency. This evidence indicates that part of the potential is dependent on respiratory energy. However, when compared to that measured under DNP containing solution (70 mV), the magnitude of potential change due to anaerobic conditions (10 mV) is rather small. This magnitude was unchanged, although the roots had adapted to the conditions for 5 hrs or more (section 6.4.2). It is likely that an alternative pathway of root respiration which takes place under oxygen deficiency (John and Greenway 1976) is responsible for the depolarization potential. Since this potential is not much different from that of A-roots, it it likely that some oxygen molecules are provided to the root under such conditions from the large air spaces in the mature cell region (see Plate 3-1), after being transferred from the shoot (Barber et al. 1962).

The appearance of the transient hyperpolarization shortly before depolarization is rather interesting, since the magnitude of the change is significant - about the same as that of depolarization. It is known that ethylene could be produced from a plastic tube (Pallaghy, personnal communication) and this can be conveyed to the root medium with the nitrogenated solution. However, this should not be the cause of the transient change, since the same tube was used to pass both aerated and nitrogenated solutions into the root medium.

It should be noted that such transient change was not only found in this study. In pea roots, Anderson et al. (1974) also observed a transient change in membrane resistance when the roots were subjected to a CN^- containing solution. Based on Ohm's Law, they suggested that the cause of this was due to an increase in transmembrane potential. In oat coleoptiles, Drake (1979) reported CN^--induced hyperpolarization which took place within 2 minutes before cells depolarized. They also showed that this was associated with a transient increase in membrane

resistance. If this is the case for rice roots, the finding of the resumption of the transient change after about 30 min in aerobic conditions could be related to time constant of the membrane.

The evidence found in this study together with those from the above workers suggests that both N_2 and CN^- can cause an decrease in ion permeability of an outer cell membrane of the root [illegible]. This effect may be a consequence of a regulation of cellular energy lavel in maintaining equal rate of energy production and utilisation (Erecinska and Wilson 1982). The cause of this transient change, however, requires further investigation. Information on some changes in mitochondria structure and enzyme activities of rice seedlings under anaerobic (nitrogen) conditions are available from Vartapetian et al. (1978) and John and Greenway (1976), respectively.

6.5.3 Consideration of the variation of membrane potential and K^+ content in aged root segments

The present study has shown that the excision caused a rapid depolarization in root segments and cell recovery, which is indicated by a gradual hyperpolarization, is taken place after about 2 hrs of ageing. The explanation for ageing -induced hyperpolarization has been related to PO_4^- and Cl^- influx (Lin and Hanson 1974a), H^+ and K^+ efflux (Smith and Harper 1977 and Gronewald and Hanson 1980), a decline of PO_4^- uptake (Bowling, Graham and Dunlop 1978 and Bowling 1983) and the sealing of plasmodesmata channels at the cut surface (Pitman et al. 1970).

Considering rice root segments, excision can cause a short-circuit between the interior and the exterior of the measuring cell, presumably via the symplast. Hence, the potential is relatively small in freshly excised segments. If cells at the cut surface are able to seal themselves (Pitman et al. 1970), the sealing should be nearly complete within 2-5 hours - the period at which hyperpolarization slowly takes place. Interestingly, this period of time coincides with the time of a marked fall of the internal K^+ concentration (chapter 5, section

5.4.2), suggesting that most of the loss of K^+ during this time was through the cut surface. However, experimental evidence in section 5.4.1 (chapter 5) suggested that a considerable amount of ions was also lost from freshly excised roots (Fig. 5.2 and 5.3) via the root surface providing that the medium was the K^+-free solution.

When a relation between ion concentration and diffusion potential is considered, using the Goldman-Hodgkin-Katz equation, the loss of K^+ ions from root segments suggested depolarization rather than hyperpolarization. This hyperpolarization is unlikely to be due to an enhanced electrogenic pump activity in the ageing roots and is presumably due to changes in the relative permeability of the membrane to ions. In other plant roots, such as corn (Mertz and Higinbotham 1976), the PD in freshly excised roots was the same as obtained from intact roots and this was associated with a steady K^+ concentration with ageing (Davis and Higinbotham 1976). In contrast to rice roots, Mertz et al. (1981) found that hyperpolarization occurred during a linear accumulation of K^+ ions in pea epicotyl segments.

It should be noted that the discrepancy in the results obtained between rice, or corn roots and pea epicotyl is not due to the tissue environments, since they were experimented in the same culture solution. If it is not the difference in tissue function, the hyperpolarization could be an indirect effect caused by movements of K^+ and other anions such as Cl^-, or cations such as H^+.

It is worth mentioning that in preliminary work, it was found that cortical cell potential of excised whole roots did not seem to differ from that of intact roots. Since the measurements in this study were made further from the cut face, the measured potential is not affected greatly by short-circuiting via the symplast. This finding together with that of excised segments suggest that root cell potentials are not directly governed by the shoot or seed. Instead, it is likely to be regulated by some activities in the root itself, and the process of regulating is likely to depend essentially on sugar and food reserves in root cells, and probably some hormones

(Pitman et al. 1970). Further supporting evidence is on the finding that transient hyperpolarization of root cells also occurred in the same fashion as appeared in intact roots when excised whole roots were subjected to oxygen deficiency.

Chapter 7

The Effect of Changes in the Oxygen State of the Root Medium on Potassium Accumulation and the Effect of Abscisic Acid on Xylem Exudate

7.1 Introduction

Life of plants under anaerobiosis has interested scientists for a number of years. Recently, Hook and Crawford (1978) have summarised work of others who provided information on some characteristics of plants grown under such conditions. Examples of these are on morphology and function, also on hormones produced and energy metabolism which are concerned more with biochemical points of view. The study in this chapter is an attempt to explain some electrophysiological aspects of the adaptation of rice roots to anaerobic conditions.

An investigation of K^+ uptake into rice roots has been made previously on the effect of change in oxygen state of the root medium by John et al. (1974). They demonstrated that the uptakes into aerobically and anaerobically grown plants were very similar. When anaerobically grown plants were transferred to a well aerated solution, there was a transient doubling of the uptake compared with roots grown under continuous aeration and the reverse was true for aerobically grown plants transferred to anaerobic conditions. Similar results have also been reported for barley seedlings (Heide et al. 1963).

It should be mentioned that the above finding in rice was true regardless of whether or not shoots were kept in the same conditions as roots. The experiments were carried out in plants of about 4 weeks old, when root morphology and the ability to adjust to the environment may differ from those of young seedlings which rely mostly on the seeds for inorganic substances. It was desirable to test whether the above results were true for young seedlings of not more than a week old.

As has been shown in the previous chapter, cortical cell potentials of anaerobically grown rice roots were smaller in magnitude than those obtained from aerobically grown ones. When the latter were transferred to anaerobic conditions, cortical cells depolarized. Since salt concentration of the

medium was the same for both aerobic and anaerobic conditions and if cell membranes did not change in ion permeability, the depolarization should be a consequence of smaller energy level available for electrogenic ion pumps than under aerobic conditions. In this respect, ionic fluxes across cell membranes when aerobic roots were transferred to anaerobic conditions could differ from those grown under continuous aerobic conditions. However, since information of ion accumulation is essential for flux estimations, the main work in this chapter was concerned with the effect of changes in the root oxygen state on the apparent influx (ϕ_{in}) by using ^{86}Rb as a tracer for K^+ ions. Part of the work dealt with tracer washout from root tissues.

A plant hormone, Abscisic acid (ABA) seems to play an important role in influencing xylem transport. There was evidence showing that it caused a rapid closure of stomata and, hence, reduced the rate of transpiration in water-stressed plants (Mittelheuser and Van Steveninck 1971, Mizrahi et al. 1970). This led to a possibility that it was involved in transport system of plant roots through the regulation of ions and water uptake into the shoots. During the past 10 years, a number of investigations on the effect of ABA on xylem transport has been made. Apparently, the effect of ABA on the transport does not appear to have a simple explanation, possibly due to an involvement of other endogenous hormones.

Pioneers of such work were Cram and Pitman (1972b) who found that xylem transport in barley roots was reduced by 50% when ABA was introduced into the root medium. Since the reduction was observed about 2 hrs before the plamalemma influx was inhibited, an active transport of ions into the xylem was concluded (also see Pitman, 1977). This conclusion, however, has been questioned on the basis that the action of ABA on the transport is a secondary effect (see discussion). Moreover, ABA showed a stimulation effect on xylem exudate in other plant species, such as maize (Collins and Kerrigan 1974), bean (Karmoker and Steveninck 1978) and sunflower (Glinka 1980). ABA-stimulated K^+ exudation in sunflower roots was reported in both high salt and low salt roots (Glinka and Abir 1983), suggesting that the salt status of the root does not determine

the ABA effect on ion transport. Although ABA does not seem to be an appropriate substance to test the active component of xylem transport, it is of interest to investigate in which respect the substance affects the transport in rice roots.

Investigations in this chapter were separated into two parts. In the first part, a study was made of the effect of changes in the oxygen state of the root medium on K^+ accumulation in root tissues to test whether young seedlings of rice were sensitive to the root environment. This experiment was carried out in both excised segments and intact roots. The second part of this chapter was concerned with the effect of ABA on ion uptake. This included both ion accumulation and exudation.

7.2 Experimental Materials

7.2.1 Preparation of A-roots and N-roots

The method of growing rice seedlings in this laboratory was described in chapter 3 (section 3.3.1). Since aeration was provided throughout plant life, they will be referred to as A-root plants. In some experiments, roots adapted to nitrogenated solution were used. They were prepared by passing N_2 gas through the root medium for 12 hours, prior to the experiment and they will be referred to as N-root plants.

All seedlings used in this chapter were 5 days old. As described before (chapter 4, section 4.3.3), they were exposed to 12 hr light and 12 hr dark for about 24 hrs prior to the experiment.

7.2.2 Abscisic acid labelled solution

Abscisic acid (ABA), (±) *cis-trans*, was used in this study. The solution was prepared in bulk by dissolving ABA in methanol to make up a stock solution of 2.5×10^{-4} M concentration, since it was known that methanol did not affect K^+ uptake in barley seedlings (Behl and Jeschke 1979). An amount of this solution was mixed with the composition of a 1x solution (chapter 3, section 3.2) to make up the required final

concentration of $5x10^{-6}$ M when used. An ABA labelled solution normally contained 2% methanol.

Note that the activities of labelled solutions used both for accumulation (section 7.3.1) and for the study of the effect of ABA on xylem exudate (section 7.3.3) were 2 μCi/ml. For tracer washout studies (section 7.3.2), the activity of 20 μCi/ml was used.

7.3 Results

7.3.1 The effect of change in root oxygen state on K^+(^{86}Rb) accumulation

The study in this section was separated into two parts, one using intact roots and the other using excised root segments. The general method for this section was as follows:

Seedlings, after being grown for 4 days, were separated into two groups; one was kept under a continuously aerated solution as in growth and the other was treated under a continuously nitrogenated solution. This separation was made about 12-15 hrs prior to the experiments and plants from both groups were grown in light on the 4th day of growing (see section 4.2.1). To avoid any effect caused by pH difference, a fresh 1x solution was used in both sets of plants. These plant roots were referred to as A-roots and N-roots, respectively (see section 7.2.1). All experiments were carried out under the same conditions as growth.

7.3.1.1 Intact roots

In order to test whether the presence of the shoot and the root tip have an influence on the adaptation of root cells to ion accumulation, experiments in this section were carried out using intact roots. Isotope loading was performed using either a 10-20 mm portion of a root (loaded horizontally) or a whole root (loaded vertically).

When the study was made in the 10-20 mm portion of the root, the apparatus shown in Fig. 4.1 was used. 5-day old

seedlings were arranged in the apparatus as described in section 4.3.1 and isotope loading was made only at the portion, either in aerated or nitrogenated solution. These solutions were prepared by passing air or N_2 gas, respectively, through the solution during tissue loading. Experiments using A-roots were designated as AA or AN, while those using N-roots were NA or NN. AA and NN were control experiments, which were carried out under the same root conditions as growth. Time for each loading in a ^{86}Rb labelled solution was 2 hrs. The method for analysing the amount of $K^+(^{86}Rb)$ in the segments was described in section 4.3.3, with the difference that the labelled roots were washed for 30 min in the K^+-free solution before being excised and only the labelled 10-20 mm portion of roots was analysed for $K^+(^{86}Rb)$.

In some experiments, whole roots were used and they were loaded vertically in a ^{86}Rb labelled solution. The level of the solution was at the seed. As the above, roots were washed in the solution for 30 min before being excised. The excision was made between 10mm and 20 mm from the tip. Segments between 0-10 mm and 10-20 mm from the tip were counted.

The results from both experiments are shown in Table 7.1(a) and (b) for horizontally labelling and vertically labelling, respectively. Numbers in the bracket represent number of plant batches/number of roots used. It can be seen that there is an indication of a smaller accumulation in AN than AA but this is not significant. In contrast to John et al. (1974), NA shows a trend of smaller accumulation than NN. A comparison between AA and NN shows that there is no difference in the accumulation. These results are true regardless of whether tissue loading was made only at the portion of the root or using the whole root.

It is interesting, however, to note that the amount of $K^+(^{86}Rb)$ accumulation in the 10-20 mm of an intact root was greater for vertical than horizontal loading. For vertically grown roots, the accumulation in the tip region (0-10 mm) is greater than that in 10-20 mm region. This suggests that the difference in the 10-20 mm root accumulation between two experiments is accounted for by a contribution from the uptake into the root tip region.

The above results clearly indicate that changes

in the root oxygen state do not greatly affect ion absorption into the roots. It is possible that the absence of any major differences in the accumulation may be due to the presence of the shoot which was in air throughout in all experiments. Some possible aspects which make these results differ from those obtained by others are discussed in section 7.4.

7.3.1.2 Excised root segments

Both A- and N-roots were excised at 20 mm from the tip. The 10-20 mm segments of each group were loaded in either aerated or nitrogenated labelled solutions for 2 hours. The method for analysing the amount of $K^+(^{86}Rb)$ in the segments was described in section 5.4.5.

A total of 5 batches of plants were investigated with 5 roots from each batch. The observations are shown in Table 7.1(a) for AA, AN, NA and NN. The accumulation of $K^+(^{86}Rb)$ in A-root and N-root tissues are in a similar pattern as appeared for intact roots, but the amount accumulated is smaller. However, it is observed that there is a significant difference between AN and NN in the accumulation of $K^+(^{86}Rb)$.

It is demonstrated in chapter 8 (section 8.4) that the K^+ inward pump at the plasmalemma does not operate in excised root segments. This could be the reason for the finding that the tissues are not sensitive to the changes in the oxygen state of the root medium, when comparing AA with AN or NA with NN. The greater accumulation in NN than in AN roots could be due to the fact than N-roots had adjusted to anaerobic conditions to some extent before being excised.

Table 7.1

Comparing $K^+(^{86}Rb)$ accumulation in a region between 0-10 mm and 10-20 mm from the root tip of A- and N-roots, under aeration (AA and NA) and nitrogenation (AN and NN) conditions of the root medium. Experiments were carried out under the same temperature as growth (25° C). Numbers in the bracket represent number of plant batches / number of roots used.

(a) In a portion of intact roots (horizontally loading)

		$K^+(^{86}Rb)$ ($m.equiv.kg^{-1}.hr^{-1}$) 10-20 mm
AA	(4/16)	.77 ± .14
AN	(4/16)	.45 ± .07
NA	(4/16)	.40 ± .03
NN	(4/16)	.57 ± .08

(b) In portions of intact roots (vertically loading)

		$K^+(^{86}Rb)$ ($m.equiv.kg^{-1}.hr^{-1}$)	
		0-10 mm	10-20 mm
AA	(3/35)	4.35 ± 1.69	1.82 ± .62
AN	(3/35)	3.12 + 0.85	1.62 ± .47
NA	(3/36)	3.64 ± 1.04	1.79 ± .02
NN	(3/40)	4.26 ± 1.53	1.83 ± .49

(c) Excised root segments

		$K^+(^{86}Rb)$ ($m.equiv.kg^{-1}.hr^{-1}$) 10-20 mm
AA	(5/25)	.44 ± .03
AN	(5/22)	.39 ± .02
NA	(5/22)	.42 ± .03
NN	(5/22)	.47 ± .02

7.3.2 Some studies of the effect of changes in the oxygen state of the root medium on tracer washout

The previous section has shown that ion accumulation in the tissue of 5-day old rice roots is not affected by the oxygen state of the root medium. Since the tissue was washed for 30 minutes before counting, the tracer retained in the sample would mainly be in cell vacuoles. The rate of long-term exchange (k_L-see section 5.4.3) might be expected to be unaffected. However, this may not be the case for the short-term component (k_s). A few experiments of tracer washout from root segments (i.e. 10-20 mm from the root tip) were carried out to observe these rates of tracer exchange. The method employed was described in section 5.4.3. ^{86}Rb was used as a tracer for K^+ ions.

It should be noted that the 4 different experiments which were AA, AN, NA and NN were carried out. The first letter refers to either A- or N-roots and the second letter to the conditions of tissue loading and washing. 5 segments from each group of plants were used in each observation.

Table 7.2 shows the values of k_s and k_L obtained from graphs of tracer remaining in the tissue with time. Averaged from 2 observations, the value of k_L, which determines mainly the vacuolar content, for AA and AN are not significantly different. This is also true when those for NA and NN are compared. k_s for AN, NN and NA are similar, while that for AA is greater than the others.

Since ion transport to the cut end of roots was not measured, estimations of ionic fluxes could not be made. However, it is envisaged that fluxes across cell membrane for each studied case should not differ from one another, since most of the ion transport into the xylem (ϕ_{cx}) was derived from the vacuole (see chapter 5, sections 5.4.1 and 5.5.1). Further evidence supporting this was from an uptake experiment using intact N-roots. It was found that tracer transport into the xylem was independent of the oxygen state of the root medium during

Table 7.2

Showing values of k_s and k_L obtained from ^{86}Rb washout from excised root segments of A- and N-roots. The first letter represents the type of roots, while the second one represents the state of loading and washing. A for aeration and N for nitrogenation.

Two observations were made under each condition with the total of 10 roots in each observation.

	k_s (hr^{-1})	k_L (hr^{-1}) $x10^{-2}$
AA	.62 ± .06	1.21 ± .10
AN	.46 ± .03	1.41 ± .34
NA	.48 ± .05	1.49 ± .01
NN	.54 ± .02	1.51 ± .18

Table 7.3

Showing K^+(^{86}Rb) accumulation in 10-20 mm root tissue and a transport of ^{86}Rb into the shoot of N-plants under aeration (NA) and nitrogenation (NN) conditions of the root medium, at different time intervals. Two observations were made, with the total of 8 plants, in each observation.

Time (hr)	Accumulation ($m.equiv.kg^{-1}$)		Transport ($cph x10^{5}$)	
	NA	NN	NA	NN
1	.27 ± .07	.49 ± .13	.27 ± .11	.39 ± .30
2	.54 ± .13	.67 ± .23	.99 ± .87	.55 ± .44
3	.73 ± .04	1.26 ± .37	.73 ± .16	1.12 ± .65
4	1.49 ± .21	1.97 ± .82	1.42 ± .21	1.45 ± .97

loading (see Table 7.3). It was expected that the result would be similar to intact A-roots and excised roots.

7.3.3 The effect of ABA on K^+(^{86}Rb) uptake

As mentioned earlier, the term "uptake" is used to represent accumulation in the root cells and transport into the xylem. This section describes how ABA affects the uptake of K^+ in excised root segments by using ^{86}Rb as a tracer. Two separate experiments were carried out, one on the accumulation effect and the other on the exudation effect. All experiments with ABA were made at 25° C, since this was found to give the maximum effect of ABA on the exudate in barley (Pitman et al. 1974b) and they were carried out in light (chapter 4). Aeration was provided throughout the experiment.

7.3.3.1 On ion accumulation

Segments between 10-20 mm from the tip of 5-day old roots were separated into two groups. One group was loaded in a ^{86}Rb labelled solution with $5x10^{-4}$ M ABA, and the other without ABA but with the standard amount of methanol. The latter was used as a control experiment to ensure that the difference in K^+(^{86}Rb) accumulation between two groups of tissue was not due to the presence of methanol. The ratio of methanol to labelled solution volume was 1:50. At the end of each time interval, segments from both groups were analysed for K^+(^{86}Rb) using the method described in 5.4.5. Four to six observations were made with a total of 16-24 roots.

The results of K^+(^{86}Rb) accumulation in the root tissue are shown in Fig. 7.1. The amount of K^+(^{86}Rb) is calculated relative to the specific activity of the external labelled solution. It shows that the accumulation in the control experiment is not significantly different from that in ABA treated tissue, over a 20 hr period.

The above result indicates that $5x10^{-4}$ M ABA has no effect on K^+(^{86}Rb) accumulation. It is possible that this concentration of ABA is too small to interact with the internal

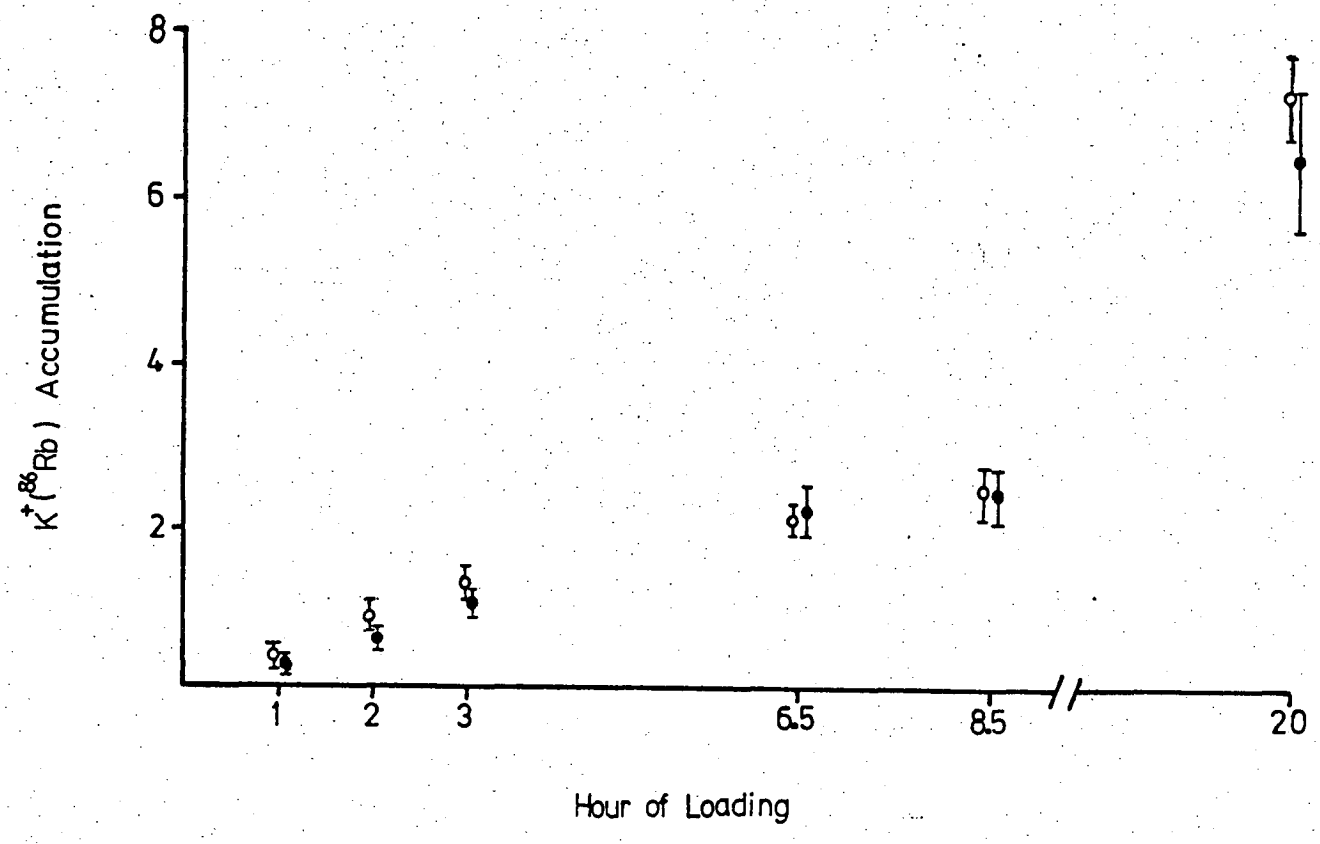


FIG. 7.1

Time course of ^{86}Rb studies of K^+ accumulation in 10-20 mm root segments. The limit is the standard eror of the mean from 4 observations for 1, 2 and 3 hr samples, 6 observations for 6.5 and 8.5 hr samples and 2 observations for 20 hr samples.

(o): control experiments and (●): with an addition of 5×10^{-4} M ABA.

substances and, consequently, no enhanced accumulation took place. Cram and Pitman (1972b) showed that Cl^- accumulation was enhanced at ABA concentrations between 10^{-7} M to 10^{-6} M, but not between 10^{-8} M and 10^{-7} M. However, Karmoker and Van Steveninck (1978) demonstrated that with a range of concentrations between $5x10^{-7}$ M to $1x10^{-4}$ M, ABA increased Na^+ and Cl^- accumulation in bean roots without affecting K^+ accumulation. Moreover, K^+ transport was enhanced by ABA at all concentrations in this range. Before a further discussion on the concentration of ABA used is made, it is of interest to investigate whether $5x10^{-4}$ M ABA has an effect on xylem transport. This is described in the following section.

7.3.3.2 On xylem exudate

Since it was difficult to collect the exudate from excised rice roots or shoots (section 4.4.6), no attempt was made to measure the volume of the exudate. Transport of K^+ into the xylem was, thus, measured by means of ^{86}Rb tracer. Since the specific activity in the symplast was not known, the content of the exudate found at the cut end was expressed as activity per kg root fresh weight. The suitability of ^{86}Rb as a tracer for K^+ ions on xylem transport was reported in chapters 4 and 5.

The apparatus shown in Fig. 5.1 was used in this experiment. 5-day old roots were excised between 5-35 mm from the tip and a portion between 15-25 mm was labelled with ^{86}Rb. The method for analysing $K^+(^{86}Rb)$ exudation was the same as described in section 5.3.2. As for the study of accumulation, the experiments were carried out in pairs, with and without $5x10^{-4}$ M ABA. The roots from both groups were allowed to adjust to the methanol in the control solution for 1 hour. The solution in the middle chamber of one experiment was then replaced with the ABA containing solution. The exudates from both experiments were collected after the same time intervals.

The results from the above experiments are shown in Fig. 7.2 which shows that the exudate for roots treated with ABA is increased within 1 hour and the increase is more pronounced at the end of 2 hours. Although only 2 experiments,

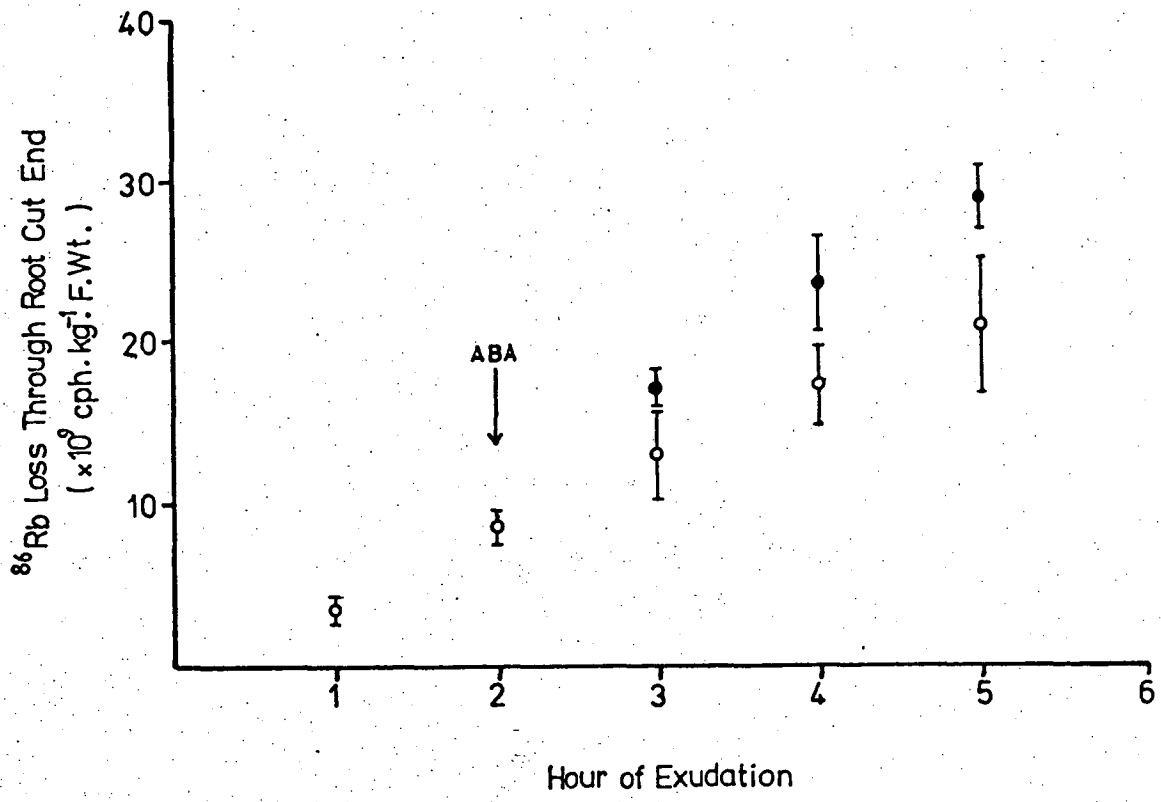


Fig. 7.2

The effect of 5×10^{-4} M ABA on ^{86}Rb exudation. The unit is expressed per kilograam fresh weight of the labelled tissue (10-20 mm from the tip). The limit is the standard error of the mean of 2 observations, with 4 roots in each.

(o): control experiments and (●): with ABA.

with 4 roots in each, were carried out, the stimulating effect of ABA on exudation from rice roots is clear. Since ^{86}Rb was found to be a suitable tracer for K^+ ions as far as transport in the symplast is concerned (chapter 4), it is concluded that ABA enhances K^+ exuadate in rice roots.

7.4 Discussion

By using ^{86}Rb as a tracer for K^+ ions, the present study demonstrated that neither excised root segments nor intact roots were sensitive to changes in the oxygen state of the root medium. When intact roots were used, the amount of tracer ions accumulated in the mature region was greater than that in excised segments, indicating that rice roots lose their absorption ability to some extent after being excised. This supports the finding of smaller fluxes for excised roots than intact roots (chapters 4 and 5). In intact roots, it was found that ion accumulation in the mature region 10-20 mm from the tip was greater when tissue loading was made using whole roots than using the portion of roots, suggesting that the root tip was a sensitive site of ion uptake.

The above results are not in agreement with the findings in 4 week old rice roots by John et al. (1974) and in one week old barley roots by Heide et al. (1963). These can be explained in view of root morphology as follows:

(a) For young seedlings, it was found that anaerobic conditions caused root enlargement in barley roots (Pitman 1969). In this study, it was observed that treating rice roots under nitrogenated solution for 12-15 hrs did not greatly affect the root size. The only affected region was that close to the root tip (see Fig. 7.3), but the increase was not significant. This could be due to a much shorter anaerobical treatment than was used for barley (i.e. 3-4 days-Pitman 1969). Besides, it is known that rice is about 10 times more effective in transporting oxygen from shoots to roots than barley (Jensen et al. 1967). The air cavities in the cortex of rice roots which were grown aerobically (chapter 3) are presumably to facilitate the oxygen transport.

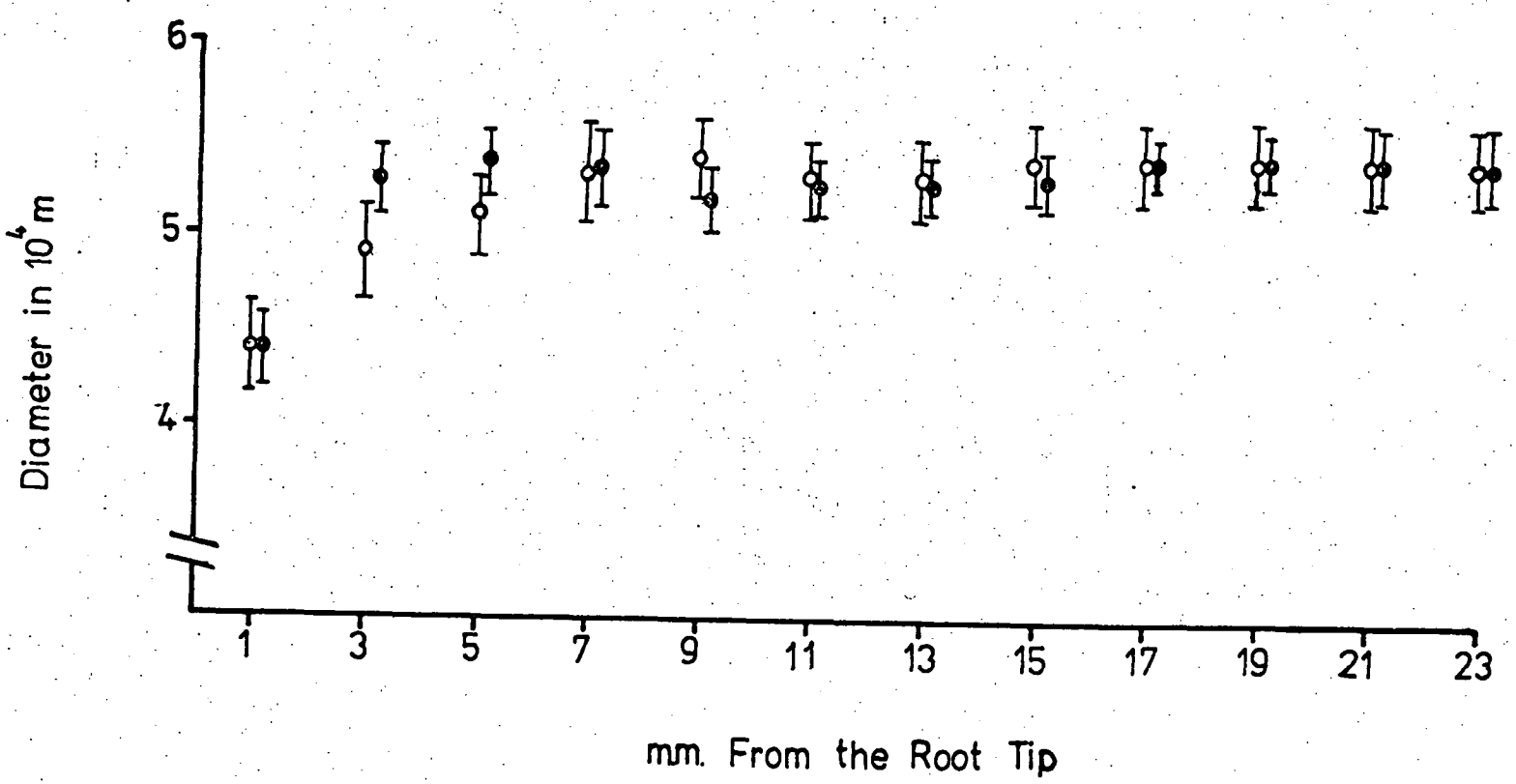


Fig. 7.3

Comparing the root diameter of 5 day old seedlings at every 2 mm from the tip to a distance of 25 mm above it. (o) represents that of A-roots and (●) of N-roots (i.e. being grown in a nitrogenated solution for 12-15 hrs on the 4th day of growing). The limit is the standard error of the mean from 10 roots.

(b) In four week old rice plants, John et al. (1974) observed that the forming of adventitious roots were more pronounced in anaerobically grown plants than in aerobically grown ones and this was accompanied by a reduction of root dry weight by about 25%. This adaptation of plants provided a larger number of root tip per unit mass than aerobically grown roots. It is most likely that the root tip is the main region for ion absorption and is the only sensitive part to the changes of the root oxygen conditions; otherwise the accumulation per unit mass for NN should be much greater than that for AA, due to the 25% mass reduction. This is reasonable, since large air spaces in the root cortex would develop closer to the root tip as a consequence of root adaptation. This is accompanied with the collapse of cortical cells (Drew et al. 1980) which makes it very unlikely that the mature region of anaerobically grown roots would absorb nutrients from the medium. The greater rate of ion uptake for NA than AA in their results is due to the smaller mass and an enhancement from root aerobic conditions. In the present study, the enhanced accumulation in NA compared to NN is not found, since in young seedlings the enhanced secondary root formation has not yet occurred. Hence, the similarity of the accumulation between these two could be due to some oxygen supply from shoots to roots during the 12-15 hrs of anaerobic treatment.

Due to the above points, it is concluded that 12-15 hr of anaerobic treatment for less than one week old seedlings was not adequate to change root morphology. For further studies, it is necessary to increase the anaerobic treatment period. Since experimental evidence suggests that the root tip is a sensitive region to the changes of the root oxygen state, the study may have to be made in this region and phloem transport must be taken into account.

On xylem exudate, this study demonstrated that 5×10^{-4} M ABA enhanced K^+ exudate of rice root segments, but had no effect on accumulation of the ions. These results were consistent with those of bean roots grown in a complete Hoagland solution for the same range of ABA concentration (Karmoker and Steveninck 1978), but contradict observations with barley and maize (Cram and Pitman 1972b, Behl and Jeschke 1979).

The effect of ABA on xylem exudate of K^+ ions in barley roots was discussed in relation to NO_3^- ions (Behl and Jeschke 1979). A permanent inhibition of the exudate was observed when experiments were carried out using NO_3^--pretreated roots in NO_3^- containing solution. With non-pretreated roots, partial recovery was found after severe inhibition of the exudate. However, this was not the case for sunflower roots. Glinka and Abir (1983) found that a stimulation effect on the exudate was independent of the ion species.

The above results in rice and other plants does imply that ABA effect on root ion accumulation is not necessarily related directly to the effect on xylem exudate. Instead, the effect may depend on the nature of the plant which is essentially determined by the level of endogeneous hormones, probably cytokinins (Pitman et al. 1974a). Evidence supporting this was given by Erlandsson et al. (1978) who demonstrated that kinetin could eliminate an ABA inhibitory effect in sunflower roots and suggested that the effect was a consequence of some alteration of ABA-kinetin balance in the root. The possibility of an ABA-induced secondary effect on xylem exudate was discussed by Lüttge and Higinbotham (1979) and Pitman (1977). Cytokinins were found in the exudate of rice roots (Murofushi et al. 1983), but it appears that no study has been performed on the presence of this hormone in relation to the effect of ABA on the exudate. This may be another interesting aspect for future work.

By compartmental analysis, Behl and Jeschke (1981a) showed that ABA concentration in excised barley roots was greater in the cytoplasm than the vacuole. Another interesting aspect is to determine the endogeneous ABA level using intact rice roots so that any alteration caused by an excess ABA or kinetin from the root medium can be followed at a compartmental level. In this case, the level of CO_2 gas surrounding the leaves may be of importance (see Blackman and Davis 1984). An interesting summary concerning the effect of hormones, such as cytokinins, gibberellins and abscisic acid, on ion transport has been provided by Van Steveninck (1976).

Chapter 8

General Conclusions

The present study appears to be the first attempt to measure ionic fluxes across intact roots which are grown under undisturbed conditions (i.e. in the same culture solution throughout their life). The Pitman model of ion transport (1971) was utilised to show that it is possible to estimate unidirectional fluxes across the mature part of the root while the root remains intact. The analytical procedure (chapter 2) can only be justified providing that fluxes are not functions of time and this is certainly not the case in young cells. The finding that symplasmic longitudinal transport between neighbouring cells is negligible, compared to the symplasmic radial transport into the xylem (chapter 4), helps to simplify the study. It is also found that, regardless of whether or not suberization at the endodermis of the mature root region is completed, apoplastic transport is only 5 % of the radial symplastic transport. This finding agrees well with Anderson (1976), Pitman (1971 and 1977) and Spanswick (1976) when roots are bathed with a low concentration solution.

For laboratory grown plants, the composition and concentration of the root medium used depend essentially on the purpose of the investigation. In normal conditions, plants such as salt bush and rice may grow well under much more concentrated solutions than some other species. This may increase the apoplastic transport to some extent so that it is no longer negligible. However, the analytical procedure for roots (chapter 2) is still valid, provided that dY_x/dt is corrected by the subtraction of $S_o\phi_{ox}$.

The present study also appears to be the first attempt to measure the efflux of ions from the xylem. It is demonstrated that re-absorption of ions from the xylem vessels into cells surrounding them (ϕ_{xc}) is at an appreciable rate (i.e. 0.40 $m.equiv.kg^{-1}.hr^{-1}$). It should be mentioned that the backflow of tracer from the xylem (i.e. $s_x\phi_{xc}$) can be negligible in the analysis even though ϕ_{xc} itself is not negligible. This is because s_x of high salt roots is small throughout the analysis. Other experimental evidence showing small s_x compared to s_o for

high salt roots was published by Davis and Higinbotham (1976).

The possiblity of re-absorption of ions from the vessels was also mentioned elsewhere (Johanson and Cheeseman 1983, Johanson et al. 1983 and Salim and Pitman 1984) without an attempt to measure it.

It should be emphasised that the main purpose of this study is to measure ionic fluxes across the mature portion of the intact root and to give a picture of how ions are moving across the root, utilising the Ussing-Teorell equation. It is further desired to compare the results obtained from the portion of intact roots with those from mature root segments, since the latter were used in most studies in the past. The results obtained from excised tip segments (i.e. 0-10 mm from the tip) can be questioned on the validity of the use of the analytical procedure.

8.1 A comparison of ion fluxes and the internal ion contents between excised root segments and intact roots

In this section, a comparison is made between the ion transport properties of a mature portion of the root both when it is excised and when it is part of an intact root. The complexities of the root tip region which contains cells in a range of stages of development will be discussed in section 8.5.

Summaries of magnitudes of fluxes and compartmental contents obtained from ^{42}K studies, from chapters 4 and 5, are shown in Fig. 8.1. Since excised root segments lose K^+ content rapidly soon after excision, the compartmental contents which are estimated during a quasi steady state of the tissue are much smaller in excised roots than in intact roots. The figure shows that most of the loss from root segments is via the plasmalemma, whereas that from intact roots is via the xylem vessels. The rate of K^+ transport into the xylem (ϕ_{cx}) is reduced by at least 80% after the excision. This is similar to the percentage of reduction of the plasmalemma influx.

Further evidence showing that root segments do not behave in the same way as intact roots comes from the study of tracer transport into the xylem (section 4.4.3 and 5.4.3). The

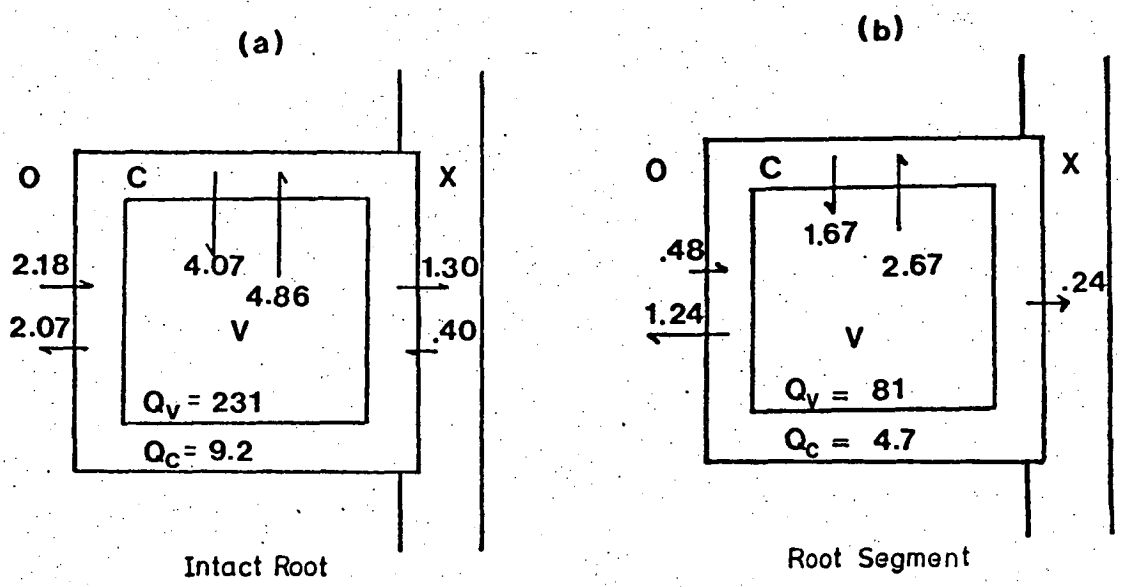


Fig. 8.1

A comparison of K^+ fluxes (half-arrows) and compartmental contents (Q_c and Q_v) between an intact root (a) and a mature root segment (b), at the same 10-20 mm distance from the tip.

O represents the external medium, C: the cytoplasm, V: the vacuole and X: the xylem vessels.

Fluxes mequiv kg^{-1} hr^{-1}
Q mequiv kg^{-1}

much smaller xylem transport in root segments than in intact roots indicates the cessation of some active functions controlling the delivery of ions into the xylem after the roots are isolated from the seeds (see next section) and, consequently, the influx at the plasmalemma becomes small. This evidence confirms the existence of a close relationship between xylem transport and the plamalemma influx. Note that excised barley roots which were grown in nutrient solutions also showed a smaller rate of transport into shoots compared to intact roots (Pitman et al. 1974b).

In intact roots, a greater net loss of K^+ from the tissue into the xylem than to the root medium indicates that cells in this region of the root and at this stage of the root development serve as a reservoir of ions for plant growth. With time, this rate of transport from the cell reserve would become smaller and lateral roots would play a major role on ion uptake for growth, particularly when K^+ in the seed becomes deficient. There is evidence in this study (chapter 3) showing that lateral roots start to form in the stele at a region between 25-30 mm from the tip.

In comparing the values of total K^+ content of root cells per kilogram fresh weight of tissue obtained from chemical assay with those from tracer analysis, one would expect a larger value from the former because that value also includes the stele. In the tracer analysis, the K^+ content is assumed to be for the cortical cells alone which occupy about 80% of the tissue volume (see Plate 3.2). In fact the two estimates were almost the same. It should be pointed out that the roots used for chemical assay were washed for 30 minutes in a K^+-free solution (with RbCl substituted for KCl) to remove free space K^+ before analysis, and subsequent studies (chapter 5) suggested that this may have enhanced K^+ loss from the tissue.

To give further insight into the transport mechanism of ions across the root cells, the Ussing-Teorell (flux ratio) equation is utilised. This equation is based on the assumption that ions are moving passively across cell membranes under electrochemical force. The ratio of the unidirectional fluxes across each membrane is determined by

$$\frac{\phi_{io}}{\phi_{oi}} = \frac{C_i}{C_o} \left[\exp\ (Z_j FE/RT) \right] \qquad (8\text{-}1)$$

where ϕ_{io} and ϕ_{oi} represent passive ion fluxes from and to the inside (i) and the outside (o) of a cell membrane; C, the concentration of the ion inside (i) and outside (o) of the membrane; Z_j, the valence of the ion species; F, the Faraday constant; E, the electropotential difference across the membrane; R, the gas constant; and T, the absolute temperature. Departures from the predictions of this equation may indicate that active transport is involved.

In order to apply this relationship to the membrane within the root, some difficulties arise because of the uncertainties in estimating certain parameters of the root. Particular difficulties include the values of cytoplasmic volume, the potential across the tonoplast and the concentration of K^+ in the xylem. It is necessary to estimate the cytoplasmic volume and the vacuolar volume before one can calculate the ion concentrations in each cell compartment.

8.2 Estimations of the cytoplasmic and vacuolar volume

A preliminary method on the attempt to estimate the cytoplasmic volume (V_c) was by observing 10μm root longitudinal sections, taken between 10-20 mm from the tip, under a 400x magnification microscope after staining the sections with Harris' haematoxylin and acid fushsin. Unlike roots of halophytes *Triglochin maritima* (Jefferies 1973), the thickness of the cytoplasm was too small to be measured.

Another approach was to assume that the cytoplasmic volume does not change with the expansion of the root cells. It can, then, be estimated by comparing the known volume of immature non-vacuolated cells to their volume when full maturation has been reached. Using this procedure, the cell was found to increase in volume by a factor of 43.3. If it is assumed that the cytoplasmic volume remained constant and that the only change is the development of the vacuole, the fraction of the total cellular volume that is cytoplasm is 1/43.3. Furthermore,

the cell occupies about 90% of the tissue volume. Hence, the cytoplasmic volume (V_c) and vacuolar volume (V_v). are 2.3% and 87.7% of total tissue volume, respectively.

The validity of estimating cytoplasmic volume by this method will be discussed later on (see section 8.3).

To determine the density of the root tissue, a range of known density alcohol solutions was prepared. Segments of roots betweem 10-20 mm from the root tip were dropped into the solutions. Suspension of the segments in any one of these solutions indicates equal density between the root tissue and the solution. To prevent entry of air into the tissue from the atmosphere, root excision was performed under the nutrient solution. It appeared that the density of the root between 10-20 mm from the tip was 0.97 of water density. These give the cytoplasmic (V_c) and the vacuolar (V_v) volume equal to 23.7 ml and 904.3 ml per kilogram tissue fresh weight, respectively.

The concentration of ion (C) in each cell compartment is related to the volume (V) and the ionic content in the compartment (Q) as

$$C = Q / V \qquad (8\text{-}2)$$

Utilising the values of Q_c and Q_v from both intact roots and excised roots (from Fig. 8.1) and the above V_c and V_v, K^+ concentration in the cytoplasm and the tonoplast designated as C_c and C_v, respectively, are 386.5 mM and 255.5 mM. The rather large value of C_c obtained by from this study will be discussed in the next section.

8.3 The application of the Ussing-Teorell equation to predict K^+ pumps for a mature portion of intact root

To predict the flux ratio by using the equation (8-1), it is assumed that the electropotential across the tonoplast is zero. This is based on the finding that there is no detectable potential difference when advancing a microelectrode from the cytoplasm to the vacuole of hair cells (chapter 6). The potential difference between the xylem and the cytoplasm of the

cortical cells cannot be obtained from intact roots since it was not possible to advance the electrode into the stele. The best estimate of this potential is, therefore, obtained from the measurement of xylem exudate from freshly excised roots whose tip was attached. Since the steady potential of the exudate was -37 mV and that of the cortical cells was -132 mV, both relative to the bathing medium (see chapter 6), the potential difference between the xylem and the cytoplasm is 95 mV, with the xylem being more positive. In order to predict the flux ratio across the xylem, the K^+ concentration of 25 mM is utilised. Further discussion on the use of this value will be included later.

Electrical potential differences, predicted flux ratios from equation (8-1) and the measured flux ratios of intact roots are shown in Table 8.1. Note that the concentration of K^+ in the medium is 1.0 mM. In common practice if the predicted ratio does not agree with the measured one, it is concluded that active transport of ions is involved in the mechanism and the magnitude and direction of the transport can be predicted. As can be seen, there is no agreement between the two values either across the plasmalemma, the tonoplast or the xylem for intact roots. Since the measured value is greater than the predicted one at the plasmalemma and the xylem, it is concluded that there is an active inward pump of K^+ ions across these boundaries. The magnitude and the direction of fluxes caused by these pumps are shown in Fig. 8.2 (a).

In contrast to the above, an active outward K^+ pump is concluded at the tonoplast. This suggests that the vacuole serves as an ion secretion organ for plant growth, the ions being accumulated in this compartment at the younger age possibly from the seed and to a lesser extent from the medium. This K^+ outward pump at the tonoplast was also inferred when the studies were made in the halophyte, *Triglochin maritima L.* by Jefferies (1973 and references therein).

The finding of the xylem inward pump in intact roots supports the two pump hypothesis, as suggested by Pitman (1972b and 1977). This pump was also inferred elsewhere by the use of inhibitors on electrical potential measurements (Kuiper

Table 8.1

A comparison between predicted (P) flux ratios across the plasmalemma (ϕ_{oc}/ϕ_{co}), the tonoplast (ϕ_{cv}/ϕ_{vc}) and the xylem (ϕ_{cx}/ϕ_{xc}) for a mature portion of an intact root, a mature root segment and a root tip segment, utilising the Ussing-Teorell equation (8-1).

C represents K^+ concentration in the cytoplasm (C), the vacuole (V) and the xylem (X) and E: transmembrane potential across the plasmalemma (E_p), the tonoplast (E_t) and the potential in the xylem exudate (E_x). M is the ratio of measured fluxes obtained from the tracer experiments. Assumed that the cytoplasmic volume was 2.3% (*) and 4.1% (**) of the tissue volume.

				Plasmalemma			Tonoplast			Xylem		
	C_c	C_v	C_x	E_p	ϕ_{oc}/ϕ_{co}		E_t	ϕ_{cv}/ϕ_{vc}		E_x	ϕ_{cx}/ϕ_{xc}	
	(mM)			(mV)	P	M	(mV)	P	M	(mV)	P	M
Intact roots*	386.5	255.5	25	-132	.44	1.05	0	1.51	.84	+95	.38	3.25
Mature root segments*	198.3	89.0	25	-113	.41	.39	0	2.23	.62	+95	.20	-
Root Tip segments**	185.3	123.2	25	-113	.44	.63	0	1.50	.71	+95	.18	-

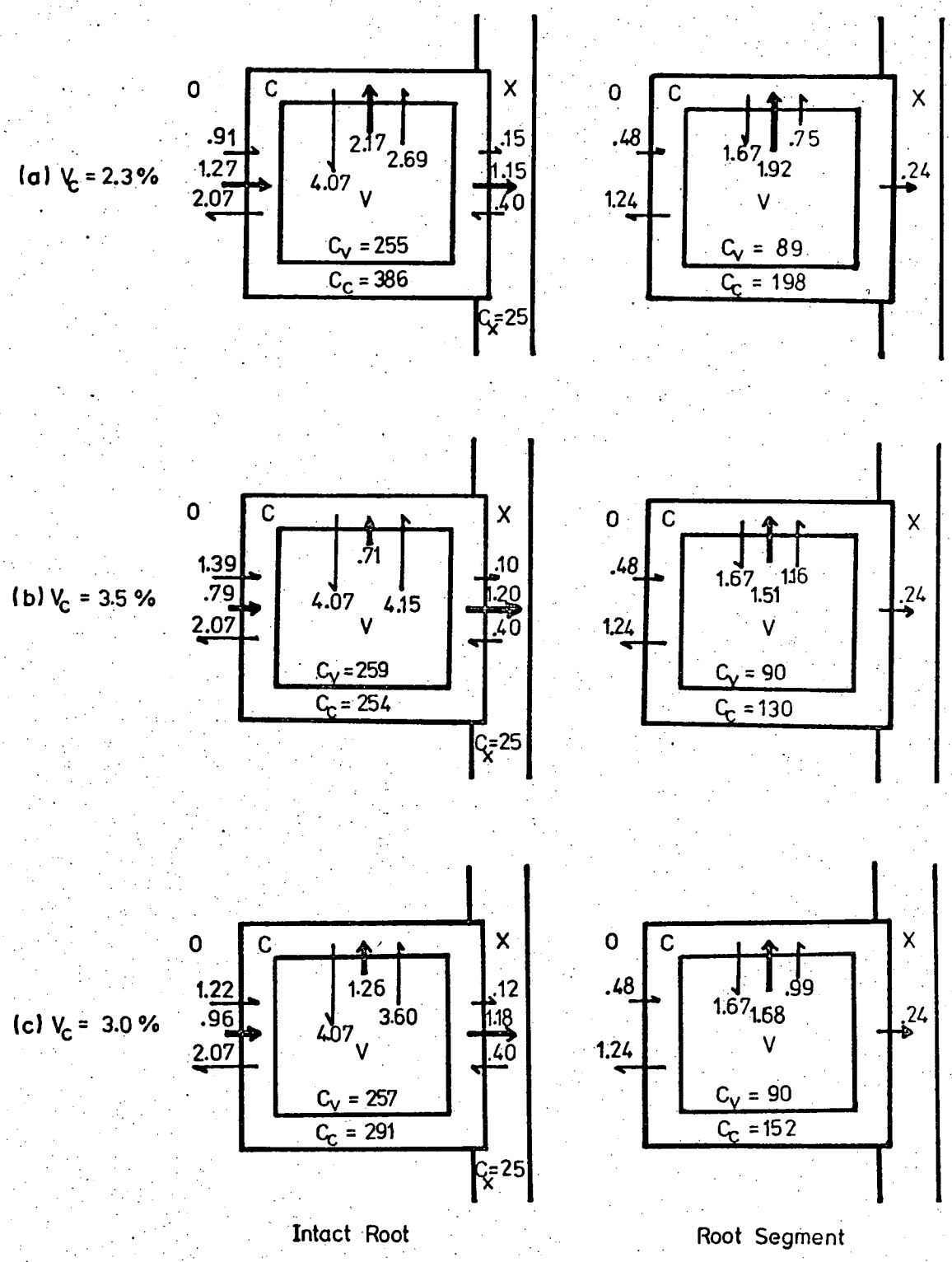


FIG. 8.2

A diagram showing passive (half-arrows) and active (thick-arrows) fluxes across the plasmalemma (ϕ_{oc} and ϕ_{co}), the tonoplast (ϕ_{cv} and ϕ_{vc}) and the xylem (ϕ_{cx} and ϕ_{xc}) and K^+ concentration in the cytoplasm (C_c), the vacuole (C_v) and the xylem (C_x) of intact roots (left column) and root segments (right column).

(a), (b) and (c) represent diagrams when the percentage of the cytoplasmic volume is changed from 2.3% to 3.5% and 3.0%, respectively, relative to the tissue volume.

Flux : mequiv kg^{-1} hr^{-1} : Q mequiv. kg^{-1}

and Boer 1980, Dunlop 1982). In the present study, it is not possible to know exactly where the xylem inward pump is located. Some scientists have suggested that the second pump should be at the xylem parenchyma cells, while others suggested it at the endodermis. Details on work related to this hypothesis are provided by Lüttge and Higinbotham (1979).

Although the estimated cytoplasmic K^+ concentration is rather high compared to other plant species, one has to remember that this study is made in intact roots and rice is generally classified as a salt tolerant plant. On osmotic grounds, the large difference in K^+ concentration between these compartments leads to speculation that there may be other cations such as Na^+ or Mg^{+2} ions to compensate in the vacuole. It was found that the combined content of Na^+ and Mg^{+2} in rice roots was about 30% of K^+ content (chapter 3). Jeschke (1977) and Jeschke et al.(1983) have discussed the possibility of Na^+/K^+ exchange across the tonoplast in barley roots, in such a way that K^+ ions in the vacuole were replaced by Na^+ ions. However, their work dealt with low salt roots which only experienced the external K^+ and Na^+ during the experimentation.

It has been considered that the high value of cytoplasmic concentration could be due to the small value of V_c assumed for these cells. It was suggested, previously, that the V_c of higher plant cells was between 3-5% of the total tissue volume (Higinbotham 1970). Nonetheless, the finding of 2.3% for V_c in the 10-20 mm region of rice roots is not unreasonable compared to the value obtained from excised 0-10 mm segments of corn roots (3.5% - Davis and Higinbotham 1976) which contain cells that are partially elongated or non-vacuolated. In the 0-10 mm region of rice roots, V_c was calculated to be 4.1% of the tissue volume. This larger percentage compared to that estimated from excised corn roots suggests that rice roots grow at a slower rate. This was found to be the case, since 5 day old corn seedlings attained 13-15 cm root length (Davis and Higinbotham 1976) while the length of rice roots which were grown in a similar solution was not greater than 5 cm (chapter 3).

Despite the above argument, another estimate of cytoplasmic K^+ concentration was made by using the cytoplasmic

volume of 3.5% of tissue volume obtained from corn roots. The calculation showed that the cytoplasmic concentration would be reduced to 254 mM and became smaller than the vacuolar concentration (259 mM). Utilising these values for the concentrations, Fig. 8.2 (b) shows that the pump mechanism at the plasmalemma and the tonoplast are still present, but that the magnitude of active fluxes becomes smaller. It is envisaged that the true value for the cytoplasmic volume of the mature cells may lie between these two estimates, about 3.0% of the total tissue volume. Utilising this V_c value, the predicted active fluxes are estimated and shown in Fig. 8.2(c). Although the various assumptions about V_c change the estimated magnitude of the pumps, their putative existence is unaffected.

Since the predicted flux ratio across the xylem was based on the most probable xylem concentration of 25 mM, one might question the validity of this value. Using the value of C_x obtained by the guttation method (i.e. 42.5 mM, section 4.4.6) and the cytoplasmic volume of 2.3%, the calculated ionic fluxes and the prediction of active fluxes are shown in diagram in Fig. 8.3 (a). As can be seen, the magnitude of the xylem active flux is greater than before (see Fig. 8.2 a). For a matter of interest, Fig. 8.3 (b) shows that if V_c of 3.5 % is used the active fluxes at the plasmalemma and the tonoplast tend to be smaller, while the xylem active flux becomes slightly greater. These indicate that the magnitude of the xylem efflux does not have a marked effect on the xylem pump.

Another argument for the existence of the xylem pump can be on the uncertainty of the xylem electopotential used, since it is obtained from excised roots. However, as mentioned earlier there is difficulty in advancing a microelectrode into the stele and the certainty of the microelectrode tip being in the xylem could be questioned (however see Dunlop 1982). If one assumes that the measured potential in the xylem sap under investigation is smaller than reality due to the possibility of a partial short-circuit between the sap and the medium along the apoplast, the positive potential of the xylem in relation to the cytoplasm should be reduced to some extent. If taking the value at the moment when the microelectrode was in contact with the sap

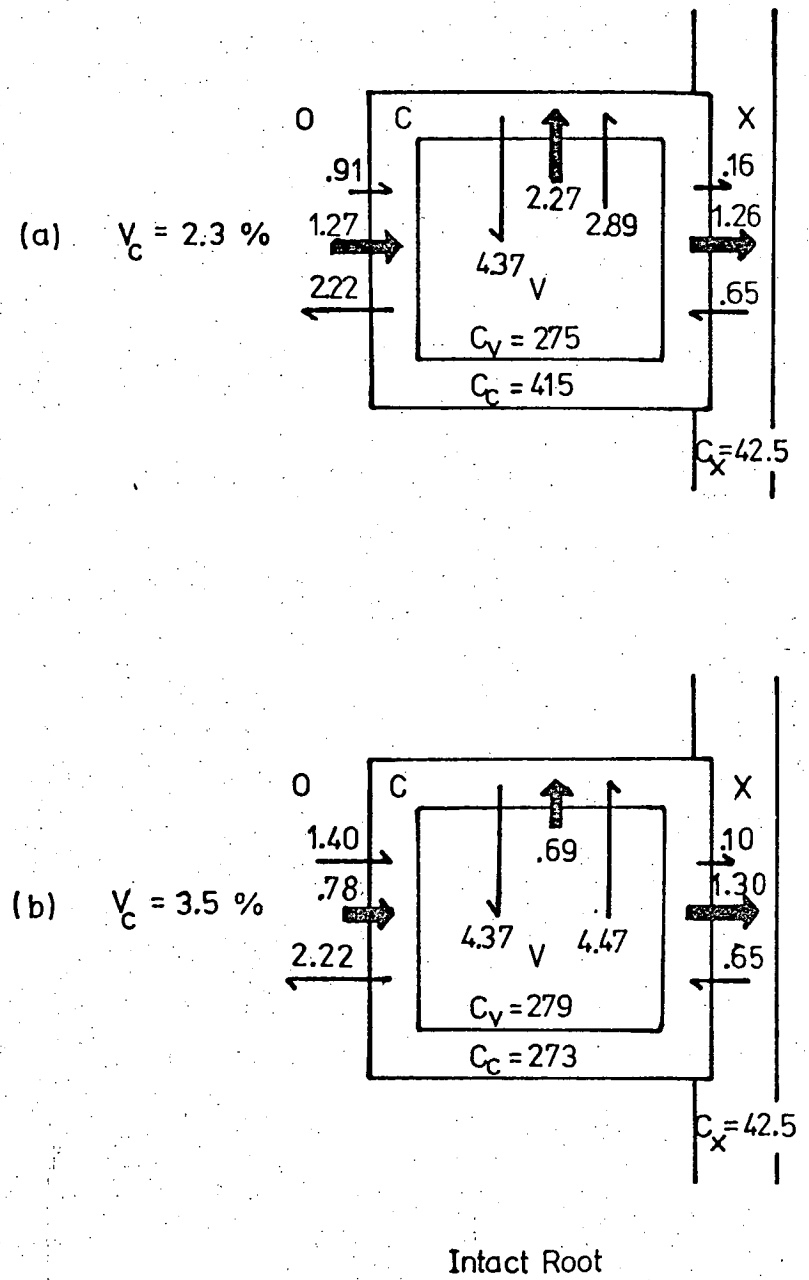


Fig. 8.3

Diagrams showing passive (half-arrows) and active (thick-arrows) fluxes across the plasmalemma (ϕ_{oc} and ϕ_{co}), the tonoplast (ϕ_{cv} and ϕ_{vc}) and the xylem (ϕ_{cx} and ϕ_{xc}) of intact roots, assuming that K^+ concentration in the xylem was 42.5 mM. Assuming the cytoplasmic volume was 2.3% (a) and 3.5 % (b) of the total tissue volume.

Flux: mequiv kg^{-1} hr^{-1} @ mequiv kg^{-1}

which was about -70 mV relative to the bathing medium (section 6.4.4), there still exists the xylem pump but the magnitude is reduced from 1.15 m.equiv.kg^{-1}.hr^{-1} to 0.75 m.equiv.kg^{-1}.hr^{-1}. This suggests that the pump is not sensitive to the magnitude of the potential.

8.4 The application of the Ussing-Teorell equation for the prediction of K^+ pumps across the mature root segments

The values of C_c and C_v used for calculation are the same as those for intact roots, since the same region of the root was studied. For zero potential across the tonoplast, the average potential across the plasmalemma taken between 5-20 hrs was -113 mV. The flux ratio equation was utilised and the results are shown in Table 8.1. Only the predicted flux ratio across the xylem was shown since O_{xc} was not measured.

Unlike intact roots, the predicted flux ratio across the outer membrane of root segments is similar to the measured one. It is, therefore, concluded that K^+ ions are moving passively across the plasmalemma. At the xylem boundary, the influx is lowered to a value close to the passive influx of the intact root. This suggests that the active inward K^+ pump into the xylem disappears after root excision. As deduced for intact roots, the active outward pump at the tonoplast remains, but is smaller in magnitude if V_c of 2.3% is used. Diagrams for the magnitude and the direction of the pump with different V_c values are shown in Fig. 8.2, in comparison to that for intact roots.

The disappearance of these pumps after root excision is likely to be due to the loss of some controls exercised on ion uptake by the shoots (Pitman 1982), and of its normal energy supplies. There have been reports concerning some controls on activity in the root by the shoot of rice seedlings, at least on an oxygen requirement (Barber et al. 1962), and it has been shown that large air spaces develop taking most of the cortex free space when the roots are fully mature (see Hoshikawa 1975). It has been also suggested that excised root cells of rice are highly sensitive to oxygen deficiency (Yoshida 1981). Although the segments were aerated throughout the experiments,

excision may have displaced some of the air pockets within the tissue, thereby reducing the oxygen availability. A further attempt was made by adding sucrose into the root medium to make up the final concentration of 1.0 mM, the results however did not provide useful information since bacterial contamination appeared surrounding the root surface, even in the case when sterilized solutions were used.

In earlier work on the mechanism of ion transport in excised roots, Higinbotham (1973) suggested that the evidence for an active K^+ pump at the plasmalemma was not conclusive. However, a comparative study using root segments and intact roots of rice suggests that whether or not the pump operates depends essentially on the state of the roots used. It is possible that in some other plants, where the pump was found, the cells are not so sensitive to being isolated from the shoot as those of rice. Alternatively, it may also depend on which region of the roots was studied since the activity of cells in the tip region may be different from that of mature cells. The following section will compare the results obtained from root tip segments with those from mature segments in order to test this.

8.5 The application of the Ussing-Teorell equation for the prediction of K^+ pumps in root tip segments

As mentioned earlier, most of flux measurement in higher plant roots was made using root tip segments and the roots were treated under a low salt condition (i.e. grown without the studied ions). So far, the work by Davis and Higinbotham (1976) using the tip segments of high salt roots of corn seems to be the only precedent. They suggested the possibility of an active inward transport of K^+ ions at the plasmalemma and into the xylem. It is a matter of interest to compare their results with those of root tip segments of rice. Since the electrical potential of cortical cells is independent of their distance from the tip in intact roots (chapter 6), it is plausible to assume the same potential exists in root tip segments as in mature root segments.

The predicted and the measured values of flux ratio across membranes of root tip segments are shown in Table 8.1, based on the value of 4.1% cytoplasmic volume relative to the tissue volume and the root density of 0.98 of water density. There is a small difference between the predicted ratio and the measured one at the plasmalemma, suggesting a weak inward K^+ pump at the boundary. Since the xylem flux appears to be about twice the passive flux for intact roots, it is possible that part of the xylem transport is due to an active process. However, no tracer studies on root tip segments were made with ^{42}K. Owing to the non-suitability of ^{86}Rb as a tracer for K^+ at the tonoplast (chapter 5), no further attempt was made to predict the transport mechanism across this membrane.

Although these results support the two pump hypothesis, it should be pointed out that the existence of the plasmalemma inward pump of excised roots depends crucially on the cortical cell PD. As is known, there have been difficulties in knowing the location of the microelectrode tip, after being advanced into the root, which leads to the uncertainty of the tonoplast potential. If the potential in the vacuole is 10 mV positive to that of the cytoplasm, as used by Davis and Higinbotham (1976), the calculation showed that there was no active inward pump at the plasmalemma.

8.6 Kinetics of ions in the xylem: A comparison between intact roots and excised roots

It has been shown in Figs. 4.7 and 5.5 that there is a lag phase of ion transport into shoots and the period is longer for the tip segments than for the mature cell region of intact roots. It was found in the present study that the longer the lag period the smaller the value of k_e. The time taken for the cytoplasm to reach the quasi-steady state is closely related to the period of the lag phase.

Considering the mature root segments and intact roots, it was observed that the specific activity in the xylem (s_x) was independant of the presence of the shoot. Table 8.2 shows the values for dY_x/dt, dQ_x/dt and s_x for these tissues. Note that dY_x/dt was obtained from experiments using ^{86}Rb as a tracer for K^+. As is seen, dY_x/dt and dQ_x/dt for intact roots are about ten times greater than those for root segments. Consequently, s_x for both tissues is about the same. Due to the larger dQ_x/dt, s_x for excised root tips is smaller than that for mature root segments.

The similarity of s_x between intact roots and root segments cannot be explained in terms of the specific activity in the symplast, since that of the former is about twice as much as the latter. Alternatively, if s_x for intact roots is diluted, due to the exposure of the root tip region to a non-labelled solution, this should also happen to root segments since a portion at the lower cut end (i.e. between 5-10 mm from the tip) was exposed to the non-labelled solution as well. However, it is expected that the rate of water flow in the xylem of intact roots should be larger than that for the segments and, subsequently, s_x for intact roots is diluted to a greater extent. Calculated from the rate of K^+ flow in the xylem (6.37×10^{-9} moles.hr^{-1} per root), the corresponding rate of water flow for intact roots is 4.25×10^{-3} (mm)^3.min^{-1}. This, however, requires a further investigation of water movement in both intact roots and root segments.

With the effect of transpiration from the shoot and some active cells at the root tip, one would expect a more

Table 8.2

Comparing the rate of ^{86}Rb transport into the xylem (dY_x/dt), the rate of K^+ transport into the xylem (dQ_x/dt) and the specific activity of the xylem (s_x) for intact roots, the mature root segments and excised root tip segments.

s_o shows the specific activity of the root medium.

	dY_x/dt cph.kg^{-1}.hr^{-1}	dQ_x/dt moles.hr^{-1}	s_o cph.mole^{-1} K^+	s_x/s_o
Intact root	61.5×10^{9}	10.85×10^{-9}	$.90\times10^{14}$	.08
Root segment	6.6×10^{9}	1.44×10^{-9}	$.73\times10^{14}$	.07
Excised tip	5.8×10^{9}	2.40×10^{-9}	$.73\times10^{14}$	.03

rapid speed of movement of K^+ in the xylem for intact roots than for root segments. By the use of ^{86}Rb isotope (see method in chapter 4), it was surprisingly found that the speed of K^+ in the xylem was 1.8±.28 mm.min^{-1}, not significantly different from that obtained from intact roots (1.7±.06 mm.min^{-1}). This finding indicates that the speed of ion transport is independent of the presence of the shoot. One may argue that the speed that was measured was not of movement in the xylem but of apoplastic or symplastic movement along the cortex. It is, however, difficult to envisage speed of this magnitude elsewhere than in the xylem. Furthermore, it would not account for the unidirectional action of the transport. Instead, the result suggests that the xylem for root segments of young seedling cannot be an inert pipe. The lower part of root segments is likely to possess living xylem in which the flow is unidirectional. Note that the measurement was made in freshly excised root segments and the experiment was carried out within 5-10 min after the excision. Therefore, cells were still active during this period.

The speed of ion transport in the xylem was also investigated by Epstein and Norlyn (1973) in excised corn roots by using a two-point application pulse-chase technique. They reported the speed of 5.9 mm.min^{-1} and 17.2 mm.min^{-1} when ^{86}Rb and ^{82}Br isotopes were used, respectively. This much larger speed compared to rice is probably due to the use of low salt roots. They also found that the speed of radial flow into the xylem was independent of the external ion concentration.

8.7 Membrane permeability coefficient for K^+ ions

The ion permeability coefficient (P_j) can be calculated from the Goldman equation in which the electric field in the membrane is assumed to be uniform. The passive unidirectional fluxes are related to P_j as

$$\phi_{oi} = -P_j \cdot \frac{Z_j F E}{RT} \cdot \frac{C_j^o}{1 - \exp(Z_j FE/RT)} \qquad (8\text{-}3)$$

$$\phi_{i,o} = P_j \cdot \frac{Z_j F E}{RT} \cdot \frac{C_{j,i} \exp(Z_j FE/RT)}{1 - \exp(Z_j FE/RT)} \qquad (8\text{-}4)$$

From cross sections of the mature root region, about 70 cortical cells are observed per cross section and each cell is 87×10^{-6} m long and 17.1×10^{-6} m wide on average. Per kg root fresh weight, the total cortical cell surface area is calculated to be 30.66 m^2. Utilising the value of passive flux at each membrane (from Fig. 8.1) together with the above surface area, K^+ permeability coefficients at the plasmalemma and the tonoplast of intact roots are 1.6×10^{-7} $cm.s^{-1}$ and 9.5×10^{-9} $cm.s^{-1}$, respectively. Thus, the tonoplast is about 16 times less permeable than the plasmalemma. In spite of this, the fluxes at the tonoplast are larger than at the plasmalemma. The smaller permeability at this membrane is due to the fact that it is bathed in both sides with concentrated K^+ solutions.

Similarly to the above, the K^+ permeability coefficients in root segments were also estimated. These values are 0.98×10^{-7} $cm.s^{-1}$ and 0.08×10^{-7} $cm.s^{-1}$ at the plasmalemma and the tonoplast, respectively. The smaller coefficients for root segments than for intact roots also reflect a decline in membrane permeability after a period of excision.

The coefficient at the plasmalemma of intact roots is comparable with that of pea epicotyl (1.1×10^{-7} $cm.s^{-1}$) and oat coleoptiles (1.7×10^{-7} $cm.s^{-1}$), under the same external K^+ concentration (Higinbotham et al. 1967), but smaller than that of broad bean roots (2.2×10^{-7} $cm.s^{-1}$, Scott et al. 1968). That for root segments is slightly greater than the root tip segments of corn (i.e. 0.56×10^{-7} $cm.s^{-1}$).

8.8 Prospects for further work

The main achievement of the present study is in the measurement of ionic fluxes using intact roots. This is another step towards providing a link between ion kinetics in the root on one hand to shoot development on the other, without having to disrupt the relationship between the two.

The evidence from these studies that there is an inward pump at the plasmalemma of rice root cortical cells is in agreement with that found in many lower plant cells (Mitchell 1961, MacRobbie 1970 and Findlay et al. 1971 and Reed and Collins 1980a and b). In lower plant cells, this active K^+ inward flux was related to an electrogenic proton extrusion mechanism (Reed et al. 1981 and Mitchell 1961). Although, an H^+ extrusion pump in high plant roots has been inferred (Spanswick 1981, Poole 1969), the relationship between H^+ and K^+ transport is not clear. This is probably because most of the study has been performed in low salt roots, and in nearly all cases the roots were excised.

The mechanism of K^+ and H^+ transport in higher plant roots were intensively studied by Cheeseman and Hanson and their co-workers. In low-salt excised corn roots, Cheeseman and Hanson (1979b) showed that K^+ flux was partially electrogenic and it was related to ATPase at the plasmalemma (Cheeseman et al. 1980). In intact roots, Cheeseman and Enkoji (1984) reported a large H^+ efflux in halophyte, *Spergularia marina* for both low salt and high salt seedlings and the efflux was sensitive to the darkness, shoot excision and fusicoccin. The evidence seems to suggest that H^+ efflux is under an active process which supported Lin and Hanson (1976) and Gronewald and Hanson (1980) on H^+/K^+ exchange. The effect of fusicoccin on K^+-in/H^+-out exchange was also discussed by Lüttge and Higinbotham (1979).

It is beyond the scope of this study to examine whether the K^+ inward pump in rice roots is electrogenic. If, as suggested by Cheeseman and Hanson, an H^+ extrusion pump exists, the K^+ inward pump cannot be electrogenic since these ions are moving in the reverse direction. It should be mentioned that the electrogenic K^+ inward pump was also suggested at the plasmalemma of algal cells with the H^+ extrusion pump, a secondary active

transport (Ritchie and Larkum, 1985).

Like other investigations mentioned earlier, the above work in intact roots dealt with the whole root system and H^+ efflux was measured during a short-term loading period. It is necessary to investigate H^+ fluxes in high-salt intact roots of rice so that a clearer picture on H^+-K^+ relationship can be revealed. Another important extension of the present studies on rice is an examination of anion kinetics. Active inward chloride transport is a vital requirement of most plant cells that have been studied (Scott et al. 1969, Higinbotham 1970, Mertz and Higinbotham 1973 and Davis and Higinbotham 1976).

Another point of view which is equally important for one to consider in a future work is the effect of aerobic and anaerobic conditions on ion transport in rice plants. For many years, a large number of scientists have been interested in the unique adaptive ability of rice to adjust to anoxic conditions (John et al. 1974, John and Greenway 1976, Johnson et al. 1978, Bertini et al. 1980, Alpi and Beevers 1983, Métraux and Kende 1984, Raskin and Kende 1984), but little is known of whether and, if so, how the ion transport mechanism is altered in order to adapt to the change in the root oxygen conditions. As demonstrated in chapter 7, K^+ uptake into root segments did not show a significant response to the change of oxygen in the root medium. Neither did the uptake into intact roots do so when the roots were subjected to anoxia. Since it is possible that young seedlings of rice are able to transfer oxygen molecules from shoots to roots efficiently (Jensen et al. 1967), a future study should be made in plants of which both the root and the shoot are subjected to anoxia.

Since the tip region of the root plays an important role in ion uptake and a number of hormones, such as abscisic acid, cytokinin and gibberellins (see Kramer 1983) are produced at the tip before being transported into the shoot, one may also be interested in comparing ion kinetics in this region of the root with that in the mature root region. Re-translocation of ions from the upper part of the plant was evidenced in this study and one should take this into account. However, it should be noted that complications may arise due to the complexity of

whole root system. The problem of the root growing during flux measurements was raised by Jeschke and Jambor (1981) when whole roots were used.

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